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(54) Title: PROTEINS AND POLYNUCLEOTIDES ENCODED THEREBY

(57) Abstract: The present invention provides novel polypeptides, termed MBSPX polypeptides, as well as polynucleotides encoding MBSPX polypeptides and antibodies that immunospecifically bind to an MBSPX or a derivative, variant, mutant, or fragment of an MBSPX polypeptide, polynucleotide or antibody. The invention additionally provides methods in which the MBSPX polypeptide, polynucleotide and antibody are used in detection and treatment of a broad range of pathological states, as well as to other uses.

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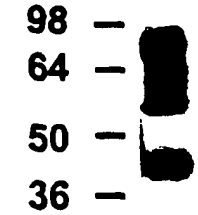

PROTEINS AND POLYNUCLEOTIDES ENCODED THEREBY

FIELD OF THE INVENTION

The invention relates in general to polynucleotides and polypeptides. The invention relates more particularly to polynucleotide sequences and the membrane-bound or secreted polypeptides encoded by such polynucleotides, as well as vectors, host cells, antibodies and recombinant methods for producing the polypeptides and polynucleotides.

BACKGROUND OF THE INVENTION

Eukaryotic cells are subdivided by membranes into multiple functionally distinct compartments that are referred to as organelles. Each organelle includes proteins essential for its proper function. These proteins can include sequence motifs often referred to as sorting signals. The sorting signals can aid in targeting the proteins to their appropriate cellular organelle(s). In addition, sorting signals can direct some proteins to be exported, or secreted, from the cell.

One type of sorting sequence is a signal sequence (also referred to as a signal peptide or leader sequence). The signal sequence is present as an amino-terminal extension on a newly synthesized polypeptide chain. A signal sequence targets proteins to an intracellular organelle called the endoplasmic reticulum (ER).

The signal peptide takes part in an array of protein-protein and protein-lipid interactions that result in translocation of a polypeptide containing the signal sequence through a channel in the ER. After translocation, a membrane-bound enzyme (signal peptidase) liberates the mature protein from the signal sequence.

The ER functions to separate membrane-bound proteins and secreted proteins from proteins that remain in the cytoplasm. Once targeted to the ER, both secreted and membrane-bound proteins can be further distributed to another cellular organelle called the Golgi apparatus. The Golgi directs the proteins to vesicles, lysosomes, the plasma membrane, mitochondria and other cellular organelles.

Only a limited number of genes encoding membrane-bound and secreted proteins have been identified. Examples of known secreted proteins include human insulin, interferon, interleukins, transforming growth factor-beta, human growth hormone, erythropoietin, and lymphokines. A need exists for identifying and characterizing additional novel human secreted proteins and the genes that encode them.

SUMMARY OF THE INVENTION

The present invention is based in part upon the discovery of novel human polynucleotide sequences and the membrane-bound or secreted polypeptides encoded by these sequences. These human nucleic acids and polypeptides encoded thereby are collectively referred to herein as "MBSPX".

Accordingly, in one aspect, the invention provides an isolated nucleic acid molecule that encodes a novel polypeptide, or a fragment, homolog, analog or derivative thereof. The nucleic acid can include, e.g., a nucleic acid sequence encoding a polypeptide at least 85% identical to a polypeptide comprising the amino acid sequences of SEQ ID NO:2n, wherein n is an integer between 1-11, or a polypeptide that is a fragment, homolog, analog or derivative thereof. The nucleic acid can include, e.g., one or more fragments from genomic DNA, or a cDNA molecule, or an RNA molecule. In particular embodiments, the nucleic acid molecule may include the sequence of any of SEQ ID NO:2n-1, wherein n is an integer between 1-11. These polypeptides and nucleic acids are related to membrane-bound or secreted proteins, as disclosed herein.

Also included in the invention is a vector containing one or more of the nucleic acids described herein, and a cell containing the vectors or nucleic acids described herein.

The invention is also directed to host cells transformed with a vector comprising any of the nucleic acid molecules described above.

In another aspect, the invention includes a pharmaceutical composition that includes an MBSPX nucleic acid and a pharmaceutically acceptable carrier or diluent.

In a further aspect, the invention includes a substantially purified MBSPX polypeptide, *e.g.*, any of the MBSPX polypeptides encoded by an MBSPX nucleic acid, and fragments, homologs, analogs, and derivatives thereof. The invention also includes a pharmaceutical

composition that includes an MBSPX polypeptide and a pharmaceutically acceptable carrier or diluent.

In a still further aspect, the invention provides an antibody that binds specifically to an MBSPX polypeptide. The antibody can be, *e.g.*, a monoclonal or polyclonal antibody, and fragments, homologs, analogs, and derivatives thereof. The invention also includes a pharmaceutical composition including MBSPX antibody and a pharmaceutically acceptable carrier or diluent. The invention is also directed to isolated antibodies that bind to an epitope on a polypeptide encoded by any of the nucleic acid molecules described above.

The invention also includes kits comprising any of the pharmaceutical compositions described above.

The invention further provides a method for producing an MBSPX polypeptide by providing a cell containing an MBSPX nucleic acid, *e.g.*, a vector that includes an MBSPX nucleic acid, and culturing the cell under conditions sufficient to express the MBSPX polypeptide encoded by the nucleic acid. The expressed MBSPX polypeptide is then recovered from the cell. Preferably, the cell produces little or no endogenous MBSPX polypeptide. The cell can be, *e.g.*, a prokaryotic cell or eukaryotic cell.

The invention is also directed to methods of identifying a MBSPX polypeptides or nucleic acids in a sample by contacting the sample with a compound that specifically binds to the polypeptide or nucleic acid, and detecting complex formation, if present.

The invention further provides methods of identifying a compound that modulates the activity of an MBSPX polypeptide by contacting MBSPX polypeptide with a compound and determining whether the MBSPX polypeptide activity is modified.

The invention is also directed to compounds that modulate MBSPX polypeptide activity identified by contacting an MBSPX polypeptide with the compound and determining whether the compound modifies activity of the MBSPX polypeptide, binds to the MBSPX polypeptide, or binds to a nucleic acid molecule encoding an MBSPX polypeptide.

In another aspect, the invention provides a method of determining the presence of or predisposition to an MBSPX-associated disorder in a subject. The method includes providing a sample from the subject and measuring the amount of MBSPX polypeptide in the subject sample. The amount of MBSPX polypeptide in the subject sample is then compared to the

amount of MBSPX polypeptide in a control sample. An alteration in the amount of MBSPX polypeptide in the subject protein sample relative to the amount of MBSPX polypeptide in the control protein sample indicates the subject has pathology related to a dysfunction in the immune system, a tissue proliferation-associated condition, or a neurological disorder. A control sample is preferably taken from a matched individual, *i.e.*, an individual of similar age, sex, or other general condition but who is not suspected of having a dysfunction in the immune system, a tissue proliferation-associated condition, or a neurological disorder. Alternatively, the control sample may be taken from the subject at a time when the subject is not suspected of having a dysfunction in the immune system, a tissue proliferation-associated condition, or a neurological disorder. In some embodiments, the MBSPX polypeptide is detected using an MBSPX antibody.

In a further aspect, the invention provides a method of determining the presence of, or predisposition to an MBSPX-associated disorder in a subject. The method includes providing a nucleic acid sample, *e.g.*, RNA or DNA, or both, from the subject and measuring the amount of the MBSPX nucleic acid in the subject nucleic acid sample. The amount of MBSPX nucleic acid sample in the subject nucleic acid is then compared to the amount of MBSPX nucleic acid in a control sample. An alteration in the amount of MBSPX nucleic acid in the sample relative to the amount of MBSPX in the control sample indicates the subject has a dysfunction in the immune system, a tissue proliferation-associated condition, or a neurological disorder.

In a still further aspect, the invention provides a method of treating or preventing or delaying an MBSPX-associated disorder. The method includes administering to a subject in which such treatment or prevention or delay is desired an MBSPX nucleic acid, an MBSPX polypeptide, or an MBSPX antibody in an amount sufficient to treat, prevent, or delay an immune disorder, a tissue proliferation-associated disorder, or a neurological disorder in the subject.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references

mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a representation of a Western blot showing expression of a protein secreted by pCEP4/Sec-10354784-transfected 293 cells.

FIG. 2 is a representation of a Western blot showing expression of a protein secreted by pCEP4/Sec-20604798-transfected 293 cells.

FIG. 3 is a representation of a Western blot showing expression of a protein secreted by pCEP4/Sec-3207791-transfected 293 cells.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides novel polypeptides and nucleotides encoded thereby. Included in the invention are eleven novel nucleic acid sequences and their encoded polypeptides. The sequences are collectively referred to as "MBSPX nucleic acids" or "MBSPX polynucleotides" and the corresponding encoded polypeptide is referred to as an "MBSPX polypeptide" or "MBSPX protein". Unless indicated otherwise, "MBSPX" is meant to refer to any of the sequences disclosed herein.

Table 1 provides a summary of the MBSPX nucleic acids and their encoded polypeptides.

Column 1 of Table 1, entitled "MBSPX No.", denotes an MBSPX number assigned to a nucleic acid according to the invention.

Column 2 of Table 1, entitled "Clone Identification Number" provides a second identification number for the indicated MBSPX.

Column 3 of Table 1, entitled "Tissue of Origin of the Clone", indicates the tissue in which the indicated MBSPX nucleic acid is expressed.

Columns 4-9 of Table 1 describe structural information as indicated for the indicated MBSPX nucleic acids and polypeptides.

Column 10 of Table 1, entitled "Protein Similarity", lists previously described proteins that are related to polypeptides encoded by the indicated MBSPX. GenBank identifiers for the previously described proteins are provided. These can be retrieved from, e.g., http://www.ncbi.nlm.nih.gov/.

Column 11 of Table 1, entitled " Signal Peptide Cleavage Site", indicates the putative amino acid position where the signal peptide is cleaved as determined by SignalP.

Column 12 of Table 1, entitled " Cellular Localization", indicates the putative cellular localization of the indicated MBSPX polypeptides.

Table 1.

MBSPX No.	Clone Identification Number	Tissue of Origin of the Clone	Nt Length	Open Reading Frame (nt)	AA Residues	Calculated Molecular Weight	Protein Similarity	Signal Peptide Cleavage Site	Cellular Localization
1	10354784.0.335	Pituitary gland	3562	728-2945	739	80202.4	ACC:O14631, human CDO; ACC:O35158 rat CDO	Between residues 25 and 26:STA-EA	Plasma membrane
2	10354784.0.335.S3347A	Pituitary gland	3562	728-2945	739	80202.4	ACC:O14631, human CDO; ACC:O35158 rat CDO	Between residues 25 and 26:STA-EA	Plasma membrane
3	17939072.0.47	Brain, adrenal gland, bone, osteosarcoma	950	83-602	173	19445.9	SPTREMBL-ACC:O00602, human ficolin	No cleavage site detected	Lysosomal lumen
4	21417374.0.9	Genomic	2523	1-2512	837	91208.5	SPTREMBL-ACC:Q62888, rat neuroligin 2	Between residues 14 and 15:GGA-QR	Plasma membrane
	3207791.0.59	Unknown	1665	77-1388	437	47136.5	None	No cleavage	Endoplasmic

5								site detected	reticulum membrane
6	3207791.0.128	Unknown	2739	670-2275	535	60290.7	None	Between residues 22 and 23:GLS-ES	Extracellular
7	3499605.0.64	Pituitary gland	681	43-463	140	16035.8	ACC:O35777, rat implantation-association protein; ACC:Q13454, human N33 protein	Between residues 29 and 30:ASA-QR	Extracellular
8	AQ013000.0.21	Unknown	670	180-471	97	11009.6	Patp:W61426, Patent application WO9824813, human keratinocyte growth factor analogue C(1,15,102)S protein; SWISSPROT-ACC:P21781, human keratinocyte growth factor precursor (FGF-7)	No cleavage site detected	Endoplasmic reticulum membrane
9	16401346.0.337	Unknown	1135	261-980	240	25397.2	None	Between residues 57 and 58:ILG-KN	Lysosomal lumen
	20604798.0.1	Unknown	2437	147-1595	483	54857.8	TREMBLNEW-	Between	Extracellular

10							ACC:AAD20029, "UNKNOWN" protein fragment	residues 33 and 34:GRA-LP	
11	27978313.0.29	Unknown	1288	733-1003	90	9358.1	SPTREMBL-ACC:Q59765, *Rhodospirillum rubrum* nicotinamide nucleotide transhydrogenase, subunit beta	Between residues 46 and 47:CWG-AT	Plasma membrane

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Table 2 provides a cross reference to the assigned MBSPX number, clone identification number and sequence identification numbers (SEQ ID NOs).

Table 2.

MBSPX No.	Clone Identification Number	SEQ ID NO Nucleic Acid	SEQ ID NO Polypeptide
1	10354784.0.335	1	2
2	10354784.0.335.S3347A	3	4
3	17939072.0.47	5	6
4	21417374.0.9	7	8
5	3207791.0.59	9	10
6	3207791.0.128	11	12
7	3499605.0.64	13	14
8	AQ013000.0.21	15	16
9	16401346.0.337	17	18
10	20604798.0.1	19	20
11	27978313.0.29	21	22

MBSPX nucleic acids and their encoded polypeptides, according to the invention, are useful in a variety of applications and contexts. The various MBSPX nucleic acids and polypeptides according to the invention are useful, *inter alia*, as novel members of protein families according to the presence of domains and sequence relatedness to previously described proteins.

For example, the MBSP1 and MBSP2 nucleic acids and their encoded polypeptides include structural motifs that are characteristic of proteins belonging to the Ig/fibronectin type

III repeat family, particularly CDO, an oncogene-, serum-, and anchorage-regulated member of the Ig/fibronectin type III repeat family. CDO is a cell adhesion molecule (CAM) of the Ig superfamily that is down-regulated by serum stimulation of confluent, quiescent cells and is constitutively down-regulated in oncogene-transformed cells. Ig/fibronectin type III family members may function as tumor suppressors, for example, loss of protein expression of at least one Ig superfamily member, DCC, in colorectal tumors is a negative prognostic marker of survival rate in patients with stage II and stage III disease. Another Ig superfamily member, C-CAM/BGP-1, is down-regulated in colorectal and prostate malignancies and ectopic restoration of its expression suppressed tumorigenicity of cell lines derived from such tumors. Thus, MBSP1 and MBSP2 nucleic acids and polypeptides, antibodies and related compounds according to the invention are useful in therapeutic applications implicated in various cancers. Furthermore, CAMs play a role in controlling cell proliferation and thereby CAMs may be involved in induction and/or maintenance of the neoplastic phenotype. Accordingly, MBSP1 and MBSP2 nucleic acids and polypeptides, antibodies and related compounds accoring to the invention are useful in the treatment of malignancies and premalignant conditions.

Members of the Ig/fibronectin type III repeat family (e.g., neural cell adhesion molecule (N-CAM), L1, F11/contactin, etc.) also participate in developmental processes in the nervous system, including cell migration, neurite extension, and axon guidance and fasciculation. DCC also functions as a receptor or component of a receptor for netrin-1, a secreted and membrane-associated protein that acts as a guidance cue for migrating axons in the developing nervous system. Thus, MBSP1 and MBSP2 nucleic acids and polypeptides, antibodies and related compounds according to the invention are useful in therapeutic application implicated in various neurological diseases, e.g., Parkinson's Disease, Alzheimer's, amyotrophic lateral sclerosis and psychiatric disorders.

MBSP3 nucleic acids and encoded polypeptides include structural motifs that are characteristic of proteins belonging to the ficolin family of proteins. Ficolins are a group of proteins containing collagen-like and fibrinogen-like (FBG) sequences and they have a similar overall structure to C1q and the collectins. Ficolins play an important role in innate immunity by binding to oligosaccharides on the surface of a variety of microbial pathogens and aiding in the initiation of phagocytosis. Accordingly, MBSP3 nucleic acids, polypeptides, antibodies

and related compounds of the invention are useful in treating dysfunctions in the immune system, e.g., autoimmune diseases, chronic inflammation, and acute inflammatory response disorders.

MBSP4 nucleic acids and encoded polypeptides include structural motifs that are characteristic of proteins belonging to the neuroligin family of proteins. Neuroligins bind to neurexins thereby mediating cell-cell interactions between neurons. Neuroligins constitute a multigene family of brain-specific proteins with distinct isoforms that function in mediating recognition processes between neurons. Thus, MBSP4 nucleic acids, polypeptides, antibodies and related compounds of the invention are useful in therapeutic applications implicated in various neurological diseases, e.g., Parkinson's Disease, Alzheimer's, amyotropic lateral sclerosis and psychiatric disorders.

MBSP5 and MBSP6 nucleic acids and their encoded polypeptides include structural motifs that are characteristic of proteins belonging to the plectin family of proteins. Plectin, an intermediate filament linking protein, is normally associated with the sarcolemma, nuclear membrane, and intermyofibrillar network in muscle, and with hemisdesmosomes in skin. Plectin is essential for the structural integrity of muscle and skin, and for normal neuromuscular transmission. Plectin plays an important role in the glial reactions after lesions. Therefore, MBSP5 and MBSP6 nucleic acids, polypeptides, antibodies and related compounds of the invention are useful in treating lesions, e.g., lesions created by wounds and strokes. Plectin is essential in maintaining the integrity of skin, skeletal muscle, and heart cytoarchitecture. Plectin deficiency may result in abnormally assembled hemidesmosomes found in basal cell carcinoma and the alteration of these adhesion structures may be the cause of peritumoral lacunae. Plectin deficiency is associated with muscular dystrophy and epidermolysis bullosa simplex (EBS), and EBS-Ogna, an autosomal dominant severe skin blistering disease. Plectin is implicated in the focal lesions typical of an autosomal recessive type of myopathy (minicore/multicore disease) and is critical for the maintenance of cardiomyocyte cytoarchitecture. Thus, MBSP5 and MBSP6 nucleic acids, polypeptides, antibodies and related compounds of the invention are useful in treating muscular dystrophy, EBS, cardiomyopathy and carcinomas.

MBSP7 nucleic acids and encoded polypeptides include structural motifs that are characteristic of the human N33 protein. N33 is a candidate tumor suppressor gene that is silenced by methylation in most colon cancer cell lines and some primary colorectal tumors. Methylation of N33 has also been shown to occur in glioblastoma multiforme. Thus, MBSP7 nucleic acids, polypeptides, antibodies and related compounds of the invention are useful as a diagnostic marker for various cancers or in the treatment of these cancers.

MBSP8 nucleic acids and encoded polypeptides include structural motifs that are characteristic of keratinocyte growth factor (KGF). Keratinocyte growth factor is a heparin-binding member of the fibroblast growth factor (FGF) family (alternatively designated FGF-7) that is expressed only in epithelial tissues from a variety of tissues. KGF is an important paracrine mediator of proliferation and differentiation in a wide variety of epithelial cells, including hepatocytes and gastrointestinal epithelial cells, type II pneumocytes, transitional urothelial cells and keratinocytes in all stratified squamous epithelia. KGF acts a cytoprotective agent against injury (e.g., radiation- and/or chemotherapy-induced injury) to epithelia and mucosa including for example, the gastrointestinal tract, lung, urinary bladder, and hair follicles. Thus, MBSP8 nucleic acids, polypeptides, antibodies and related compounds of the invention are useful in therapeutic applications for treatment of inflammation, for repair of various tissues and organs and for treatment of human epithelial malignancies.

MBSP11 nucleic acids and encoded polypeptides include structural motifs that are characteristic of nicotinamide nucleotide transhydrogenase family of proteins. The energy-transducing nicotinamide nucleotide transhydrogenases are membrane-bound enzymes that catalyze the direct transfer of a hydride ion between NAD(H) and NADP(H) in a reaction that is coupled to transmembrane proton translocation. Tumor cells often exist in an ischemic microenvironment that would compromise the growth of normal cells. To minimize intracellular acidification under these conditions, these cells may upregulate hydrogen ion transport mechanisms. Accordingly, MBSP11 nucleic acids, polypeptides, antibodies and related compounds of the invention are useful in treating ischemic conditions and various cancers, including malignant breast cancer and solid tumors.

1. MBSP1. CLONE 10354784.0.335, A NOVEL MEMBER OF THE IG/FIBRONECTIN TYPE III REPEAT FAMILY

An MBSP1 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of clone 10354784.0.335.

A polynucleotide of the present invention has been identified as clone 10354784.0.335. 10354784.0.335 is a full-length clone of 3562 nucleotides, including the entire coding sequence of a protein from nucleotides 728 to 2945 (also referred to herein as "10354784.0.335 protein"). The clone was originally obtained from pituitary gland tissues.

The nucleotide sequence of 10354784.0.335 is reported in SEQ ID NO:1. The predicted amino acid sequence of the 10354784.0.335 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:2.

The nucleotide sequence disclosed herein for 10354784.0.335 was searched against the GenBank databases using BLASTN search protocols. The BLASTN search showed 71% identities (450 over 632 nucleotides) to human CDO mRNA, 3986 bp (GenBank Accession No:AF004841).

Searches in publicly available GenBank database BLASTP showed 50% identities (265/525) with ACC:O35158 rat CDO protein (1256 aa) and 50% identities (259/518) with ACC:O14631 human CDO (1240 aa). CDO is an oncogene-, serum-, and anchorage-regulated member of the Ig/fibronectin type III repeat family (Kang et al., J. Cell Biol. 138 (1), 203-213 (1997)). Based upon homology, 10354784.0.335 proteins and each homologous protein or peptide may share at least some activity.

10354784.0.335 was searched against other databases using SignalPep and PSort search protocols. 10354784.0.335 seems to have a cleavable amino terminal signal peptide with a cleavage site between positions 25 and 26 (STA-EA). The protein is most likely located in the plasma membrane. The predicted molecular weight is 80202.4 daltons.

The 10354784.0.335 nucleic acid and encoded polypeptide have the following sequences:

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1 GTATCATTTTCCATTCTTTTTGGGGCCTCCGAAACTGTATAAATT

46 TCAGGTTTTAGAAAACCTGGGTGTGTCCCTGGTTGGCATATAAAG
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91 CGGAATCACACATAGTCCCCTTGCTCCTTGAAGGTTGCTGAGGAA

136 CGGCACACATTAGAGAGTAAACAGGCCTTTCAGTGAGTTCTCTGC

181 AGTTTGTCCACAGTGTTGAAAAAAGATTACAGCTTTCCCAGCTGT

226 GCACCTGAGGAAGTACATAGGTGATTTGCATTTGGGGACCTTGCA

271 ATATGAGAAATGCATGTGTTTAAACAGTGGATTCCATTCAGCTCA

316 GCCGGAGGCCGGCTCTGAGATGCTCACTGAGAGACAGTTGGGCCT

361 GAGAACCATAGGGTGGGGTTGAGAGCATGGCAGATTCTTGTTTCC

406 CATCTCATCTTCAGCCTCACAGCGCACATACTGAGTGCAAGCAGA

451 AAGAAATATCTGTACCATTTAAACTGCCTCTACACTCCCTCACCT

496 TTCTCTCTTTGCCAGCACACAGTTAACTGTGCATATGTTATGTTG

541 ATGCTGCTGTTCTTCTGTGTTATCTCATTTCTTACTCATAACAGC

586 TCCCTGCAGAAGCAGTCCTTGTTTCTGATAAGGACACCAAGCCCC

631 AAGGGAATTCTGTAGCACGCCCCACTCTACATAGGTTGAAAGACC

676 CGGAATGGCTGTTTGATCCCATCTCCATGCTCTCTGGGACTGCCT

721 CCTGGGCATGCTCTACNAGGACATCCTGGTNNNCCACACGCCTTC

          MetLeuTyr---AspIleLeuVal---HisThrProSe

766 TGTCCTTGCCCTCCTTGCCCCTCCAGGCTCCACCGCTGAGGCTGC

    rValLeuAlaLeuLeuAlaProProGlySerThrAlaGluAlaAl

811 CCGCATCATCTACCCCCCAGAGGCCCAAACCATCATTGTCACCAA

    aArgIleIleTyrProProGluAlaGlnThrIleIleValThrLy

856 AGGCCAGAGTCTCATTCTGGAGTGTGTGGCCAGTGGAATCCCACC

    sGlyGlnSerLeuIleLeuGluCysValAlaSerGlyIleProPr

901 CCCACGGGTCACCTGGGCCAAGGATGGGTCCAGTGTCACCGGCTA

    oProArgValThrTrpAlaLysAspGlySerSerValThrGlyTy

946 CAACAAGACGCGCTTCCTGCTGAGCAACCTCCTCATCGACACCAC

    rAsnLysThrArgPheLeuLeuSerAsnLeuLeuIleAspThrTh

991 CAGCGAGGAGGACTCAGGCACCTCCCGGTGCATGCCCGACAATGG

    rSerGluGluAspSerGlyThrSerArgCysMetProAspAsnGl

1036 GGTTGGGCAGCCCGGGGCAGCGGTCATCCTCTACAATGTCCAGGT

     yValGlyGlnProGlyAlaAlaValIleLeuTyrAsnValGlnVa
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1081 GTTTGAACCCCCTGAGGTCACCATGGAGCTATCCCAGCTGGTCAT
     lPheGluProProGluValThrMetGluLeuSerGlnLeuValIl
1126 CCCCTGGGGCCAGAGTGCCAAGCTTACCTGTGAGGTGCGTGGGAA
     eProTrpGlyGlnSerAlaLysLeuThrCysGluValArgGlyAs
1171 CCCCCCGCCCTCCGTGCTGTGGCTGAGGAATGCTGTGCCCCTCAT
     nProProProSerValLeuTrpLeuArgAsnAlaValProLeuIl
1216 CTCCAGCCAGCGCCTCCGGCTCTCCCGCAGGGCCCTGCGCGTGCT
     eSerSerGlnArgLeuArgLeuSerArgArgAlaLeuArgValLe
1261 CAGCATGGGGCCTGAGGACGAAGGCGTCTACCAGTGCATGGCCGA
     uSerMetGlyProGluAspGluGlyValTyrGlnCysMetAlaGl
1306 GAACGAGGTTGGGAGCGCCCATGCCGTAGTCCAGCTGCGGACCTC
     uAsnGluValGlySerAlaHisAlaValValGlnLeuArgThrSe
1351 CAGGCCAAGCATAACCCCAAGGCTATGGCAGGATGCTGAGCTGGC
     rArgProSerIleThrProArgLeuTrpGlnAspAlaGluLeuAl
1396 TACTGGCACACCTCCTGTATCACCCTCCAAACTCGGCAACCCTGA
     aThrGlyThrProProValSerProSerLysLeuGlyAsnProGl
1441 GCAGATGCTGAGGGGGCAACCGGCGCTCCCCAGACCCCCAACGTC
     uGlnMetLeuArgGlyGlnProAlaLeuProArgProProThrSe
1486 AGTGGGGCCTGCTTCCCCGCAGTGTCCAGGAGAGAAGGGGCAGGG
     rValGlyProAlaSerProGlnCysProGlyGluLysGlyGlnGl
1531 GGCTCCCGCCGAGGCTCCCATCATCCTCAGCTCGCCCCGCACCTC
     yAlaProAlaGluAlaProIleIleLeuSerSerProArgThrSe
1576 CAAGACAGACTCATATGAACTGGTGTGGCGGCCTCGGCATGAGGG
     rLysThrAspSerTyrGluLeuValTrpArgProArgHisGluGl
1621 CAGTGGCCGGGCGCCAATCCTCTACTATGTGGTGAAACACCGCAA
     ySerGlyArgAlaProIleLeuTyrTyrValValLysHisArgLy
1666 GGTCACAAATTCCTCTGACGATTGGACCATCTCTGGCATTCCAGC
     sValThrAsnSerSerAspAspTrpThrIleSerGlyIleProAl
1711 CAACCGGCACCGCCTGACCCTCACCAGACTTGACCCCGGGAGCTT
     aAsnArgHisArgLeuThrLeuThrArgLeuAspProGlySerLe
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1756 GTATGAAGTGGAGATGGCAGCTTACAACTGTGCGGGAGAGGGCCA

uTyrGluValGluMetAlaAlaTyrAsnCysAlaGlyGluGlyGl

1801 GACAGCCATGGTCACCTTCCGAACTGGACGGCGGCCCAAACCCGA

nThrAlaMetValThrPheArgThrGlyArgArgProLysProGl

1846 GATCATGGCCAGCAAAGAGCAGCAGATCCAGAGAGACGACCCTGG

uIleMetAlaSerLysGluGlnGlnIleGlnArgAspAspProGl

1891 AGCCAGTCCCCAGAGCAGCAGTCCCCAGAGCAGCAGCCAGCCAGA

yAlaSerProGlnSerSerSerProGlnSerSerSerGlnProAs

1936 CCACGGCCGCCTCTCCCCCCCCAGAAGCTCCCGACAGGCCCACCAT

pHisGlyArgLeuSerProProGluAlaProAspArgProThrIl

1981 CTCCACGGCCTCCGAGACCTCAGTGTACGTGACCTGGATTCCCCG

eSerThrAlaSerGluThrSerValTyrValThrTrpIleProAr

2026 TGGGAATGGTGGGTTCCCAATCCAGTCCTTCCGTGTGGAGTACAA

gGlyAsnGlyGlyPheProIleGlnSerPheArgValGluTyrLy

2071 GAAGCTAAAGAAAGTGGGAGACTGGATTCTGGCCACCAGCGCCAT

sLysLeuLysLysValGlyAspTrpIleLeuAlaThrSerAlaIl

2116 CCCCCCATCGCGGCTGTCCGTGGAGATCACGGGCCTAGAGAAAGG

eProProSerArgLeuSerValGluIleThrGlyLeuGluLysGl

2161 AGCCTCCTACAAGTTTCGAGTCCGGGCTCTGAACATGCTGGGGGA

yAlaSerTyrLysPheArgValArgAlaLeuAsnMetLeuGlyGl

2206 GAGCGAGCCCAGCGCCCCCTCTCGGCCCTACGTGGTGTCGGGCTA

uSerGluProSerAlaProSerArgProTyrValValSerGlyTy

2251 CAGCGGTCGCGTGTACGAGAGGCCCGTGGCAGGTCCTTATATCAC

rSerGlyArgValTyrGluArgProValAlaGlyProTyrIleTh

2296 CTTCACGGATGCGGTCAATGAGACCACCATCATGCTCAAGTGGAT

rPheThrAspAlaValAsnGluThrThrIleMetLeuLysTrpMe

2341 GTACATCCCAGCAAGTAACAACAACACCCCAATCCATGGCTTTTA

tTyrIleProAlaSerAsnAsnAsnThrProIleHisGlyPheTy

2386 TATCTATTATCGACCCACAGACAGTGACAATGATAGTGACTACAA

rIleTyrTyrArgProThrAspSerAspAsnAspSerAspTyrLy

2431 GAAGGATATGGTGGAAGGGGACAAGTACTGGCACTCCATCAGCCA

sLysAspMetValGluGlyAspLysTyrTrpHisSerIleSerHi

2476 CCTGCAGCCAGAGACCTCCTACGACATTAAGATGCAGTGCTTCAA

sLeuGlnProGluThrSerTyrAspIleLysMetGlnCysPheAs

2521 TGAAGGAGGGGAGAGCGAGTTCAGCAACGTGATGATCTGTGAGAC

nGluGlyGlyGluSerGluPheSerAsnValMetIleCysGluTh

2566 CAAAGCTCGGAAGTCTTCTGGCCAGCCTGGTCGACTGCCACCCCC

rLysAlaArgLysSerSerGlyGlnProGlyArgLeuProProPr

2611 AACTCTGGCCCCACCACAGCCGCCCCTTCCTGAAACCATAGAGCG

oThrLeuAlaProProGlnProProLeuProGluThrIleGluAr

2656 GCCGGTGGGCACTGGGGCCATGGTGGCTCGCTCCAGCGACCTGCC

gProValGlyThrGlyAlaMetValAlaArgSerSerAspLeuPr

2701 CTATCTGATTGTCGGGGTCGTCCTGGGCTCCATCGTTCTCATCAT

oTyrLeuIleValGlyValValLeuGlySerIleValLeuIleIl

2746 CGTCACCTTCATCCCCTTCTGCTTGTGGAGGGCCTGGTCTAAGCA

eValThrPheIleProPheCysLeuTrpArgAlaTrpSerLysGl

2791 AAAACATACAACAGACCTGGGTTTTCCTCGAAGTGCCCTTCCACC

nLysHisThrThrAspLeuGlyPheProArgSerAlaLeuProPr

2836 CTCCTGCCCGTATACTATGGTGCCATTGGGAGGACTCCCAGGCCA

oSerCysProTyrThrMetValProLeuGlyGlyLeuProGlyHi

2881 CCAGGCAGTGGACAGCCCTACCTCAGTGGCATCAGTGGACGGGCC

sGlnAlaValAspSerProThrSerValAlaSerValAspGlyPr

2926 TGTGCTAATGGGATCCACATGAATAGGGGCTGCCCCTCGGCTGCA

oValLeuMetGlySerThr (SEQ ID NO:2)

2971 GTGGGCTACCCGGGCATGAAGCCCCAGCAGCACTGCCCAGGCGAG

3016 CTTCAGCAGCAGAGTGACACCAGCAGCCTGCTGAGGCAGACCCAT

3061 CTTGGCAATGGATATGACCCCCAAAGTCACCAGATCACGAGGGGT

3106 CCCAAGTCTAGCCCGGACGAGGGCTCTTTCTTATACACACTGCCC

3151 GACGACTCCACTCACCAGCTGCTGCAGCCCCATCACGACTGCTGC

3196 CAACGCCAGGAGCAGCCTGCTGCTGTGGGCCAGTCAGGGGTGAGG

3241 AGAGCCCCCGACAGTCCTGTCCTGGAAGCAGTGTGGGACCCTCCA

3286 TTTCACTCAGGGCCCCCATGCTGCTTGGGCCTTGTGCCAGTTGAA

3331 GAGGTGGACAGTCCTGACTCCTGCCAAGTGAGTGGAGGAGACTGG

3376 TGTCCCCAGCACCCCGTAGGGGCCTACGTAGGACAGGAACCTGGA

3421 ATGCAGCTCTCCCCGGGGCCACTGGTGCGTGTGTCTTTTGAAACA

3466 CCACCTCTCACAATTTAGGCAGAAGCTGATATCCCAGAAAGACTA

3511 TATATTGTTTTTTTTTTAAAAAAAAAAAAAAAAAAACCCCGGGGGC

3556 GGGCCCC (SEQ ID NO:1)

2. MBSP2. CLONE 10354784.0.335.S3347A, A NOVEL MEMBER OF THE IG/FIBRONECTIN TYPE III REPEAT FAMILY

An MBSP2 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of clone 10354784.0.335.S3347A.

Clone 10354784.0.335.S3347A is a novel member of the Ig/fibronectin type III repeat family. A polynucleotide of the present invention has been identified as clone 10354784.0.335.S3347A. 10354784.0.335.S3347A is a full-length clone of 3562 nucleotides, including the entire coding sequence of a protein from nucleotides 728 to 2945 (also referred to herein as "10354784.0.335 protein"). The clone was originally obtained from pituitary gland tissues.

The nucleotide sequence of 10354784.0.335.S3347A is reported below. The predicted amino acid sequence of the 10354784.0.335.S3347A protein corresponding to the foregoing nucleotide sequence is reported below.

The nucleotide sequence of related sequence 10354784.0.335 (disclosed in copending U. S. Ser. No. 60/159,231, filed Oct. 13, 1999) was searched against the GenBank databases using BLASTN search protocols. The BLASTN search showed that sequence 10354784.0.335 has 71% identity (450 over 632 nucleotides) to human CDO mRNA, 3986 bp (GenBank Accession No:AF004841).

Searches in publicly available GenBank database BLASTP showed that the protein encoded in related sequence 10354784.0.335 has 50% identity (265/525) with ACC:O35158 rat CDO protein (1256 aa) and 50% identities (259/518) with ACC:O14631 human CDO

(1240 aa). CDO is an oncogene-, serum-, and anchorage-regulated member of the Ig/fibronectin type III repeat family (Kang et al., J. Cell Biol. 138 (1), 203-213 (1997)). Based upon homology, 10354784.0.335 and 10354784.0.335.S3347A proteins and each homologous protein or peptide may share at least some activity.

Related polypeptide sequence 10354784.0.335 was searched against other databases using SignalPep and PSort search protocols. 10354784.0.335 seems to have a cleavable amino terminal signal peptide with a cleavage site between positions 25 and 26 (STA-EA). The protein is most likely located in the plasma membrane. The predicted molecular weight of protein 10354784.0.335 is 80202.4 daltons.

The 10354784.0.335.S3347A nucleic acid has the following sequence:

GTATCATTTTCCATTCTTTTTGGGGCCTCCGAAACTGTATAAATTTCAGGTTTTAGAAAACCTGGGTGTGTCCCTGGTTG

8 GCATATAAAGCGGAATCACACATAGTCCCCTTGCTCCTTGAAGGTTGCTGAGGAACGGCACACATTAGAGAGTAAACAGG

16 CCTTTCAGTGAGTTCTCTGCAGTTTGTCCACAGTGTTGAAAAAAGATTACAGCTTTCCCAGCTGTGCACCTGAGGAAGTA

24 CATAGGTGATTTGCATTTGGGGACCTTGCAATATGAGAAATGCATGTGTTTAAACAGTGGATTCCATTCAGCTCAGCCGG

32 AGGCCGGCTCTGAGATGCTCACTGAGAGACAGTTGGGCCTGAGAACCATAGGGTGGGGTTGAGAGCATGGCAGATTCTTG

40 TTTCCCATCTCATCTTCAGCCTCACAGCGCACATACTGAGTGCAAGCAGAAAGAAATATCTGTACCATTTAAACTGCCTC

48 TACACTCCCTCACCTTTCTCTCTTTGCCAGCACACAGTTAACTGTGCATATGTTATGTTGATGCTGCTGTTCTTCTGTGT

56 TATCTCATTTCTTACTCATAACAGCTCCCTGCAGAAGCAGTCCTTGTTTCTGATAAGGACACCAAGCCCCAAGGGAATTC

64 TGTAGCACGCCCCACTCTACATAGGTTGAAAGACCCGGAATGGCTGTTTGATCCCATCTCCATGCTCTCTGGGACTGCCT

72 CCTGGGCATGCTCTACNAGGACATCCTGGTNNNCCACACGCCTTCTGTCCTTGCCCTCCTTGCCCCTCCAGGCTCCACCG

80 CTGAGGCTGCCCGCATCATCTACCCCCCAGAGGCCCAAACCATCATCGTCACCAAAGGCCAGAGTCTCATTCTGGAGTGT

88 GTGGCCAGTGGAATCCCACCCCCACGGGTCACCTGGGCCAAGGATGGGTCCAGTGTCACCGGCTACAACAAGATGCGCTT

96 CCTGCTGAGCAACCTCCTCATCGACACCACCAGCGAGGAGGACTCAGGCACCTACCGCTGCATGGCCGACAATGGGGTTG

104 GGCAGCCCGGGGCAGCGGTCATCCTCTACAATGTCCAGGTGTTTGAACCCCCTGAGGTCACCATGGAGCTATCCCAGCTA

112 GTCATCCCCTGGGGCCAGAGTGCCAAGCTTACCTGTGAGGTGCGTGGGAACCCCCCGCCCTCCGTGCTGTGGCTGAGGAA

120 TGCTGTGCCCCTCATCTCCAGCCAGCGCCTCCGGCTCTCCCGCAGGGCCCTGCGCGTGCTCAGCATGGGGCCTGAGGACG

128 AAGGCGTCTACCAGTGCATGGCCGAGAACGAGGTTGGGAGCGCCCATGCCGTAGTCCAGCTGCGGACCTCCAGGCCAAGC

136 ATAACCCCAAGGCTATGGCAGGATGCTGAGCTGGCTACTGGCACACCTCCTGTATCACCCTCCAAACTCGGCAACCCTGA

144 GCAGATGCTGAGGGGGCAACCGGCGCTCCCCAGACCCCCAACGTCAGTGGGGCCTGCTTCCCCGCAGTGTCCAGGAGAGA

152 AGGGGCAGGGGGCTCCCGCCGAGGCTCCCATCATCCTCAGCTCGCCCCGCACCTCCAAGACAGACTCATATGAACTGGTG

160 TGGCGGCCTCGGCATGAGGGCAGTGGCCGGGCGCCAATCCTCTACTATGTGGTGAAACACCGCAAGGTCACAAATTCCTC

168 TGACGATTGGACCATCTCTGGCATTCCAGCCAACCGGCACCGCCTGACCCTCACCAGACTTGACCCCGGGAGCTTGTATG

176 AAGTGGAGATGGCAGCTTACAACTGTGCGGGAGAGGGCCAGACAGCCATGGTCACCTTCCGAACTGGACGGCGGCCCAAA

184 CCCGAGATCATGGCCAGCAAAGAGCAGCAGATCCAGAGAGACGACCCTGGAGCCAGTCCCCAGAGCAGCAGTCCCCAGAG

192 CAGCAGCCAGCCAGACCACGGCCGCCTCTCCCCCCCAGAAGCTCCCGACAGGCCCACCATCTCCACGGCCTCCGAGACCT

200 CAGTGTACGTGACCTGGATTCCCCGTGGGAATGGTGGGTTCCCAATCCAGTCCTTCCGTGTGGAGTACAAGAAGCTAAAG

208 AAAGTGGGAGACTGGATTCTGGCCACCAGCGCCATCCCCCCATCGCGGCTGTCCGTGGAGATCACGGGCCTAGAGAAAGG

216 AGCCTCCTACAAGTTTCGAGTCCGGGCTCTGAACATGCTGGGGGAGAGCGAGCCCAGCGCCCCCTCTCGGCCCTACGTGG

224 TGTCGGGCTACAGCGGTCGCGTGTACGAGAGGCCCGTGGCAGGTCCTTATATCACCTTCACGGATGCGGTCAATGAGACC

232 ACCATCATGCTCAAGTGGATGTACATCCCAGCAAGTAACAACAACACCCCAATCCATGGCTTTTATATCTATTATCGACC

240 CACAGACAGTGACAATGATAGTGACTACAAGAAGGATATGGTGGAAGGGGACAAGTACTGGCACTCCATCAGCCACCTGC

248 AGCCAGAGACCTCCTACGACATTAAGATGCAGTGCTTCAATGAAGGAGGGGAGAGCGAGTTCAGCAACGTGATGATCTGT

256 GAGACCAAAGCTCGGAAGTCTTCTGGCCAGCCTGGTCGACTGCCACCCCCAACTCTGGCCCCACCACAGCCGCCCCTTCC

264 TGAAACCATAGAGCGGCCGGTGGGCACTGGGGCCATGGTGGCTCGCTCCAGCGACCTGCCCTATCTGATTGTCGGGGTCG

272 TCCTGGGCTCCATCGTTCTCATCATCGTCACCTTCATCCCCTTCTGCTTGTGGAGGGCCTGGTCTAAGCAAAAACATACA

280 ACAGACCTGGGTTTTCCTCGAAGTGCCCTTCCACCCTCCTGCCCGTATACTATGGTGCCATTGGGAGGACTCCCAGGCCA

288 CCAGGCAGTGGACAGCCCTACCTCAGTGGCATCAGTGGACGGGCCTGTGCTAATGGGATCCACATGAATAGGGGCTGCCC

296 CTCGGCTGCAGTGGGCTACCCGGGCATGAAGCCCCAGCAGCACTGCCCAGGCGAGCTTCAGCAGCAGAGTGACACCAGCA

304 GCCTGCTGAGGCAGACCCATCTTGGCAATGGATATGACCCCCAAAGTCACCAGATCACGAGGGGTCCCAAGTCTAGCCCG

312 GACGAGGGCTCTTTCTTATACACACTGCCCGACGACTCCACTCACCAGCTGCTGCAGCCCCATCACGACTGCTGCCAACG

320 CCAGGAGCAGCCTGCTGCTGTGGGCCAGTCAGGGGTGAGGAGAGCCCCCGACAGTCCTGTCCTGGAAGCAGTGTGGGACC

328 CTCCATTTCACTCAGGGCCCCCATGCTGCTTGGGCCTTGTGCCAGTTGAAGAGGTGGACAGTCCTGACTCCTGCCAAGTG

336 AGTGGAGGAGACTGGTGTCCCCAGCACCCCGTAGGGGCCTACGTAGGACAGGAACCTGGAATGCAGCTCTCCCCGGGGCC

344 ACTGGTGCGTGTGTCTTTTGAAACACCACCTCTCACAATTTAGGCAGAAGCTGATATCCCAGAAAGACTATATATTGTTT

352 TTTTTTTAAAAAAAAAAAAAAAAAAACCCCGGGGGGGGGCCCC (SEQ ID NO:3)

The 10354784.0.335.S3347A encoded polypeptide has the following sequence:

1 MLYXDILVXHTPSVLALLAPPGSTAEAARIIYPPEAQTIIVTKGQSLILECVASGIPPPRVTWAKDGSSVTGYNKMRFLL

81 SNLLIDTTSEEDSGTYRCMADNGVGQPGAAVILYNVQVFEPPEVTMELSQLVIPWGQSAKLTCEVRGNPPPSVLWLRNAV

161 PLISSQRLRLSRRALRVLSMGPEDEGVYQCMAENEVGSAHAVVQLRTSRPSITPRLWQDAELATGTPPVSPSKLGNPEQM

241 LRGQPALPRPPTSVGPASPQCPGEKGQGAPAEAPIILSSPRTSKTDSYELVWRPRHEGSGRAPILYYVVKHRKVTNSSDD

321 WTISGIPANRHRLTLTRLDPGSLYEVEMAAYNCAGEGQTAMVTFRTGRRPKPEIMASKEQQIQRDDPGASPQSSSPQSSS

401 QPDHGRLSPPEAPDRPTISTASETSVYVTWIPRGNGGFPIQSFRVEYKKLKKVGDWILATSAIPPSRLSVEITGLEKGAS

481 YKFRVRALNMLGESEPSAPSRPYVVSGYSGRVYERPVAGPYITFTDAVNETTIMLKWMYIPASNNNTPIHGFYIYYRPTD

561 SDNDSDYKKDMVEGDKYWHSISHLQPETSYDIKMQCFNEGGESEFSNVMICETKARKSSGQPGRLPPPTLAPPQPPLPET

641 IERPVGTGAMVARSSDLPYLIVGVVLGSIVLIIVTFIPFCLWRAWSKQKHTTDLGFPRSALPPSCPYTMVPLGGLPGHQA

721 VDSPTSVASVDGPVLMGST (SEQ ID NO:4)

This protein sequence represents an ORF also found in 10354784.0.335.

3. MBSP3. CLONE 17939072.0.47

An MBSP3 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of clone 17939072.0.47.

A polynucleotide of the present invention has been identified as clone 17939072.0.47. 17939072.0.47 is a full-length clone of 950 nucleotides, including the entire coding sequence of a protein from nucleotides 83 to 602 (also referred to herein as "17939072.0.47 protein"). The clone was originally obtained from different tissues, including brain, adrenal gland, bone and osteosarcoma.

The nucleotide sequence of 17939072.0.47 is reported in SEQ ID NO:5. The predicted amino acid sequence of the 17939072.0.47 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:6.

The nucleotide sequence disclosed herein for 17939072.0.47 was searched against the GenBank databases using BLASTN search protocols. The BLASTN search showed 65% homology (161 of 246 nucleotides) to mouse adipocyte-specific mRNA, 1725 bp (GenBank-Accession Number M61737); 59% identity (272 of 240 nucleotides) to mouse angiopoietin 3 (Ang3) mRNA, 1530 bp (GenBank-Accession Number AF113707); and); 64% identity (169 of 264 nucleotides) to mouse ficolin B mRNA, 919 bp (GenBank-Accession Number AF063217)

Searches in publicly available GenBank database BLASTP with the amino acid sequence of 173 amino acids showed 53% identities (48 of 90 amino acids) to human ficolin, 326 amino acids (SPTREMBL-ACC:O00602). Based upon homology, 17939072.0.47

proteins and each homologous protein or peptide may share at least some activity.

The nucleotide sequence and amino acid sequence disclosed herein for 17939072.0.47 was searched against other databases using SignalPep and PSort search protocols. 17939072.0.47 does not seem to have a amino terminal signal peptide. The protein is most likely located in the lysosomal lumen. The predicted molecular weight is 19445.9 daltons.

The 17939072.0.47 nucleic acid and encoded polypeptide have the following sequences:

```
1 CTAGAATTCAGCGGCCGCTGAATTCTAGTGCAGAGTGAGCAAGGG

46 CCGCCTCATCCAGCTTCTCTCTGAGAGCCAGGGCCACATGGCTCA

                                          MetAlaHi

91 CCTGGTGAACTCCGTCAGCGACATCCTGGATGCCCTGCAGAGGGA

   sLeuValAsnSerValSerAspIleLeuAspAlaLeuGlnArgAs

136 CCGGGGGCTGGGCCGGCCCCGCAACAAGGCCGACCTTCAGAGAGC

    pArgGlyLeuGlyArgProArgAsnLysAlaAspLeuGlnArgAl

181 GCCTGCCCGGGGAACCCGGCCCCGGGGCTGTGCCACTGGCTCCCG

    aProAlaArgGlyThrArgProArgGlyCysAlaThrGlySerAr

226 GCCCCGAGACTGTCTGGACGTCCTCCTAAGCGGACAGCAGGACGA

    gProArgAspCysLeuAspValLeuLeuSerGlyGlnGlnAspAs

271 TGGCGTCTACTCTGTCTTTCCCACCCACTACCCGGCCGGCTTCCA

    pGlyValTyrSerValPheProThrHisTyrProAlaGlyPheGl

316 GGTGTACTGTGACATGCGCACGGACGGCGGCGGCTGGACGGTGTT

    nValTyrCysAspMetArgThrAspGlyGlyGlyTrpThrValPh

361 TCAGCGCCGGGAGGACGACTCCGTGAACTTCTTCCGGGGCTGGGA

    eGlnArgArgGluAspAspSerValAsnPhePheArgGlyTrpAs

406 TGCGTACCGAGACGGCTTTGGCAGGCTCACCGGGGAGCACTGGCT

    pAlaTyrArgAspGlyPheGlyArgLeuThrGlyGluHisTrpLe

451 AGGGCTCAAGAGGATCCACGCCTGGCTAGGGCTCAAGAGGATCCA

    uGlyLeuLysArgIleHisAlaTrpLeuGlyLeuLysArgIleHi

496 CGTCTGGCTAGGGCTCAAGAGGATCCACGCTCAACTGGTAGTGGC

    sValTrpLeuGlyLeuLysArgIleHisAlaGlnLeuValValAl
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541 CACAAGCCTGGCAGCTGTAGAGCCGCTAACCTCCCGACACCTCCC
    aThrSerLeuAlaAlaValGluProLeuThrSerArgHisLeuPr
586 TCACCACACAGGACCCTGAGTGAGGAGGAGGGGCTGGAAACCTGG
    oHisHisThrGlyPro  (SEQ ID NO:6)
631 GATGGGTTGGCCAAAGGAGAACCTCAGGCTCCTGGCCTGGCCCAG
676 CTCCTTCCTGCCCAAGGTAGCTTAGCCCATCCAGACTGGTCCTGA
721 AGTCTGTCCCTCCATTGGCATGAAGTCTGCCCCTCAGCAGTCCGG
766 CCTCACAGGCTGTACTTTCATGGTGCTCTCTACCTTCTGGCCCCC
811 ATCCCAGAACATTCGTGAGTGAATTCGCAAGCATACTAGCATGTG
856 ATATTAGGGAGTTTGCAATAAATTATTGATGCTGATGTAGAAAAA
901 AAAAGCTTTCAGTGAAGATGGCAGGGCCAGAACTGTTGCTTGACT
946 CCAAC  (SEQ ID NO:5)
```

4. MBSP4. CLONE 21417374.0.9, HUMAN NEUROLIGIN 2 HOMOLOG

An MBSP4 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of clone 21417374.0.9.

A polynucleotide of the present invention has been identified as clone 21417374.0.9. 21417374.0.9 is a full-length clone of 2523 nucleotides, including the entire coding sequence of a secreted protein from nucleotides 1 to 2512 (also referred to herein as "21417374.0.9 protein"). The clone was originally obtained from placenta.

The nucleotide sequence of 21417374.0.9 is reported in SEQ ID NO:7. The predicted amino acid sequence of the 21417374.0.9 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:8.

The nucleotide sequence disclosed herein for 21417374.0.9 was searched against the GenBank databases using BLASTN search protocols. The BLASTN search showed 91% identity (2311 of 2523 nucleotides) to rat neuroligin 2 mRNA, 3993 bp (GenBank-Accession Number U41662). The predicted amino acid sequence of 837 amino acids was searched in the publicly available GenBank database BLASTP and is 97% identical (813 of 837 amino acids) to the sequence of rat neuroligin 2 (SPTREMBL-ACC:Q62888). Based upon homology,

21417374.0.9 proteins and each homologous protein or peptide may share at least some activity.

The nucleotide sequence and amino acid sequence disclosed herein for 21417374.0.9 was searched against other databases using SignalPep and PSort search protocols. 21417374.0.9 seems to have a cleavable amino terminal signal peptide with a cleavage site between positions 14 and 15 (GGA-QR). The protein is most likely located in the plasma membrane. The predicted molecular weight is 91208.5 daltons.

The 21417374.0.9 nucleic acid and encoded polypeptide have the following sequences:

```
1 ATGTGGCTCCTGGCGCTGTGTCTGGTGGGGCTGGGCGGGGCTCAA
  MetTrpLeuLeuAlaLeuCysLeuValGlyLeuGlyGlyAlaGln
46 CGCGGGGGAGGGGGTCCCGCGGCGGCGCCCCCGGGCGGCCCCGGC
   ArgGlyGlyGlyGlyProAlaAlaAlaProProGlyGlyProGly
91 CTGGGCCTCGGCAGCCTCGGCGAGGAGCGCTTCCCGGTGGTGAAC
   LeuGlyLeuGlySerLeuGlyGluGluArgPheProValValAsn
136 ACGGCCTACGGGCGAGTGCGCGGTGTGCGGCGCGAGCTCAACAAC
    ThrAlaTyrGlyArgValArgGlyValArgArgGluLeuAsnAsn
181 GAGATCCTGGGCCCCGTCGTGCAGTTCTTGGGCGTGCCCTACGCC
    GluIleLeuGlyProValValGlnPheLeuGlyValProTyrAla
226 ACGCCGCCCCCTGGGCGCCCGCCGCTTCCAGCCGCCTGAGGCGCCC
    ThrProProLeuGlyAlaArgArgPheGlnProProGluAlaPro
271 GCCTCGTGGCCCGGCGTGCGCAACGCCACCACCCTGCCGCCCGCC
    AlaSerTrpProGlyValArgAsnAlaThrThrLeuProProAla
316 TGCCCGCAGAACCTGCACGGGGCGCTGCCCGCCATCATGCTGCCT
    CysProGlnAsnLeuHisGlyAlaLeuProAlaIleMetLeuPro
361 GTGTGGTTCACCGACAACTTGGAGGCGGCCGCCACCTACGTGCAG
    ValTrpPheThrAspAsnLeuGluAlaAlaAlaThrTyrValGln
406 AACCAGAGCGAGGACTGCCTGTACCTCAACCTCTACGTGCCCACC
    AsnGlnSerGluAspCysLeuTyrLeuAsnLeuTyrValProThr
451 GAGGACGGTCCGCTCACAAAAAAACGTGACGAGGCGACGCTCAAT
    GluAspGlyProLeuThrLysLysArgAspGluAlaThrLeuAsn
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496 CCGCCAGACACAGATATCCGTGACCCTGGGAAGAAGCCTGTGATG
    ProProAspThrAspIleArgAspProGlyLysLysProValMet
541 CTGTTTCTCCATGGCGGCTCCTACATGGAGGGGACCGGAAACATG
    LeuPheLeuHisGlyGlySerTyrMetGluGlyThrGlyAsnMet
586 TTCGATGGCTCAGTCCTGGCTGCCTATGGCAACGTCATTGTAGCC
    PheAspGlySerValLeuAlaAlaTyrGlyAsnValIleValAla
631 ACGCTCAACTACCGTCTTGGGGTGCTCGGTTTTCTCAGCACCGGG
    ThrLeuAsnTyrArgLeuGlyValLeuGlyPheLeuSerThrGly
676 GACCAGGCTGCAAAAGGCAACTATGGGCTCCTGGACCAGATCCAG
    AspGlnAlaAlaLysGlyAsnTyrGlyLeuLeuAspGlnIleGln
721 GCCCTGCGCTGGCTCAGTGAAAACATCGCCCACTTTGGGGGCGAC
    AlaLeuArgTrpLeuSerGluAsnIleAlaHisPheGlyGlyAsp
766 CCCGAGCGTATCACCATCTTTGGTTCCGGGGCAGGGGCCTCCTGC
    ProGluArgIleThrIlePheGlySerGlyAlaGlyAlaSerCys
811 GTCAACCTTCTGATCCTCTCCCACCATTCAGAAGGGCTGTTCCAG
    ValAsnLeuLeuIleLeuSerHisHisSerGluGlyLeuPheGln
856 AAGGCCATCGCCCAGAGTGGCACCGCCATTTCCAGCTGGTCTGTC
    LysAlaIleAlaGlnSerGlyThrAlaIleSerSerTrpSerVal
901 AACTACCAGCCGCTCAAGTACACGCGGCTGCTGGCAGCCAAGGTG
    AsnTyrGlnProLeuLysTyrThrArgLeuLeuAlaAlaLysVal
946 GGCTGTGACCGAGAGGACAGTGCTGAAGCTGTGGAGTGTCTGCGC
    GlyCysAspArgGluAspSerAlaGluAlaValGluCysLeuArg
991 CGGAAGCCCTCCCGGGAGCTGGTGGACCAGGACGTGCAGCCTGCC
    ArgLysProSerArgGluLeuValAspGlnAspValGlnProAla
1036 CGGTACCACATCGCCTTTGGGCCCGTGGTGGATGGCGACGTGGTC
     ArgTyrHisIleAlaPheGlyProValValAspGlyAspValVal
1081 CCCGATGACCCTGAGATCCTCATGCAGCAGGGAGAATTCCTCAAC
     ProAspAspProGluIleLeuMetGlnGlnGlyGluPheLeuAsn
1126 TACGACATGCTCATCGGTGTCAACCAGGGAGAGGGCCTCAAGTTC
     TyrAspMetLeuIleGlyValAsnGlnGlyGluGlyLeuLysPhe
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1171 GTGGAGGACTCTGCAGAGAGCGAGGACGGTGTGTCTGCCAGCGCC
     ValGluAspSerAlaGluSerGluAspGlyValSerAlaSerAla
1216 TTTGACTTCACTGTCTCCAACTTTGTGGACAACCTGTATGGCTAC
     PheAspPheThrValSerAsnPheValAspAsnLeuTyrGlyTyr
1261 CCGGAAGGCAAGGATGTGCTTCGGGAGACCATCAAGTTTATGTAC
     ProGluGlyLysAspValLeuArgGluThrIleLysPheMetTyr
1306 ACAGACTGGGCCGACCGGGACAATGGCGAAATGCGCCGCAAAACC
     ThrAspTrpAlaAspArgAspAsnGlyGluMetArgArgLysThr
1351 CTGCTGGCGCTCTTTACTGACCACCAATGGGTGGCACCAGCTGTG
     LeuLeuAlaLeuPheThrAspHisGlnTrpValAlaProAlaVal
1396 GCCACTGCCAAGCTGCACGCCGACTACCAGTCTCCCGTCTACTTT
     AlaThrAlaLysLeuHisAlaAspTyrGlnSerProValTyrPhe
1441 TACACCTTCTACCACCACTGCCAGGCGGAGGGCCGGCCTGAGTGG
     TyrThrPheTyrHisHisCysGlnAlaGluGlyArgProGluTrp
1486 GCAGATGCGGCGCACGGGGATGAACTGCCCTATGTCTTTGGCGTG
     AlaAspAlaAlaHisGlyAspGluLeuProTyrValPheGlyVal
1531 CCCATGGTGGGTGCCACCGACCTCTTCCCCTGTAACTTCTCCAAG
     ProMetValGlyAlaThrAspLeuPheProCysAsnPheSerLys
1576 AATGACGTCATGCTCAGTGCCGTGGTCATGACCTACTGGACCAAC
     AsnAspValMetLeuSerAlaValValMetThrTyrTrpThrAsn
1621 TTCGCCAAGACTGGGGACCCCAACCAGCCGGTGCCGCAGGATACC
     PheAlaLysThrGlyAspProAsnGlnProValProGlnAspThr
1666 AAGTTCATCCACACCAAGCCCAATCGCTTCGAGGAGGTGGTGTGG
     LysPheIleHisThrLysProAsnArgPheGluGluValValTrp
1711 AGCAAATTCAACAGCAAGGAGAAGCAGTATCTGCACATAGGCCTG
     SerLysPheAsnSerLysGluLysGlnTyrLeuHisIleGlyLeu
1756 AAGCCACGCGTGGCGGACAACTACCGCGCCAACAAGGTGGCCTTC
     LysProArgValAlaAspAsnTyrArgAlaAsnLysValAlaPhe
1801 TGGCTGGAGCTCGTGCCCCACCTGCACAACCTGCACACGGAGCTC
     TrpLeuGluLeuValProHisLeuHisAsnLeuHisThrGluLeu
```

1846 TTCACCACCACCACGCGCCTGCCTCCCTACGCCACGCGCTGGCCG

PheThrThrThrThrArgLeuProProTyrAlaThrArgTrpPro

1891 CCTCGTCCCCCGCGGCGTGGCGCCCCGGGCACACGCCGGCCCCCG

ProArgProProArgArgGlyAlaProGlyThrArgArgProPro

1936 CCGCCTGCCACCCTGCCTCCCGAGCCCGAGCCCGAGCCCGGCCCA

ProProAlaThrLeuProProGluProGluProGluProGlyPro

1981 AGGGCCTATGACCGCTTCCCCGGGGACTCACGGGACTACTCCACG

ArgAlaTyrAspArgPheProGlyAspSerArgAspTyrSerThr

2026 GAGCTGAGCGTGACCGTGGCCGTGGGTGCCTCCCTCCTCTTCCTC

GluLeuSerValThrValAlaValGlyAlaSerLeuLeuPheLeu

2071 AACATCCTGGCCTTTGCTGCCCTCTACTACAAGCGGGACCGGCGG

AsnIleLeuAlaPheAlaAlaLeuTyrTyrLysArgAspArgArg

2116 CAGGAGCTGCGGTGCAGGCGGCTTAGCCCACGTGGCGGCTCAGGC

GlnGluLeuArgCysArgArgLeuSerProArgGlyGlySerGly

2161 TCTGGCGTGCCTGGTGGGGGCCCCCTGCTCCCCGCCGCGGGCCGT

SerGlyValProGlyGlyGlyProLeuLeuProAlaAlaGlyArg

2206 GAGCTGCCACCAGAGGAGGAGCTGGTGTCACTGCAGCTGAAGCGG

GluLeuProProGluGluGluLeuValSerLeuGlnLeuLysArg

2251 GGTGGTGGCGTCGGGGCGGACCCTGCCGAGGCTCTGCGCCCTGCC

GlyGlyGlyValGlyAlaAspProAlaGluAlaLeuArgProAla

2296 TGCCCGCCCGACTACACCCTGGCCCTGCGCCGGGCACCGGACGAT

CysProProAspTyrThrLeuAlaLeuArgArgAlaProAspAsp

2341 GTGCCTCTCTTGGCCCCGGGGGCCCTGACCCTGCTGCCCAGTGGC

ValProLeuLeuAlaProGlyAlaLeuThrLeuLeuProSerGly

2386 CTGGGGCCACCGCCACCCCCACCGCCCCCCTCCCTGCTGCAGATC

LeuGlyProProProProProProProSerLeuLeuGlnIle

2431 TTCGGGCCCTTCCCCCCGCCCCCTCCCACCGCCACCAGCCACAAC

PheGlyProPheProProProProProThrAlaThrSerHisAsn

2476 AACACGCTACCCCACCCCCACTCCACCACTCGGGTATAGGGGGTG

AsnThrLeuProHisProHisSerThrThrArgVal (SEQ ID NO:8)

2521 GGT (SEQ ID NO:7)

5. MBSP5. CLONE 3207791.0.59, A NOVEL PROTEIN

An MBSP5 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of clone 3207791.0.59.

A polynucleotide of the present invention has been identified as clone 3207791.0.59. 3207791.0.59 is a full-length clone of 1665 nucleotides, including the entire coding sequence of a protein from nucleotides 77 to 1388 (also referred to herein as "3207791.0.59 protein").

The nucleotide sequence of 3207791.0.59 is reported in SEQ ID NO:9. The predicted amino acid sequence of the 3207791.0.59 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:10.

The nucleotide sequence disclosed herein for 3207791.0.59 was searched against the GenBank databases using BLASTN search protocols. The BLASTN search showed 98% homology (505 of 513 nucleotides at the 3' end) to human clone A9A2BRB7 (CAC)n/(GTG)n repeat-containing mRNA, 1047 bp (GenBank-Accession Number U00952). The nucleotide sequence showed also 56% identitities (362 of 641 nucleotides) to the human gene for plectin, 14189 bp (GENBANK-acc:Z54367).

Searches in publicly available GenBank database BLASTP using the amino acid sequence of 437 amino acids showed no significant homologies to known proteins.

The nucleotide sequence and amino acid sequence disclosed herein for 3207791.0.59 was searched against other databases using SignalPep and PSort search protocols. 3207791.0.59 does not seem to have an amino terminal signal peptide. The protein is most likely located in the endoplasmic reticulum membrane. The predicted molecular weight is 47136.5 daltons.

The 3207791.0.59 nucleic acid and encoded polypeptide have the following sequences:

1 CAACAAGTTAAGCTGAAGACCGAAGCAAGAGCTGGTTCAGGTGGC

46 AGCCACAGCAGCCTCAGGGACCTCAGCAACTATGGCCTCCTGCCC

MetAlaSerCysPr

```
91 AGACTCTGATAATAGCTGGGTGCTTGCTGGCTCCGAGAGCCTGCC
   oAspSerAspAsnSerTrpValLeuAlaGlySerGluSerLeuPr
136 AGTGGAGACACTGGGCCCGGCATCCAGGATGGACCCAGAATCTGA
    oValGluThrLeuGlyProAlaSerArgMetAspProGluSerGl
181 GAGAGCCCTGCAGGCCCCTCACAGCCCCTCCAAGACAGATGGGAA
    uArgAlaLeuGlnAlaProHisSerProSerLysThrAspGlyLy
226 AGAATTAGCTGGGACCATGGATGGAGAAGGGACGCTCTTCCAGAC
    sGluLeuAlaGlyThrMetAspGlyGluGlyThrLeuPheGlnTh
271 TGAAAGCCCTCAGTCTGGCAGCATTCTAACAGAGGAGACTGAGGT
    rGluSerProGlnSerGlySerIleLeuThrGluGluThrGluVa
316 CAAGGGCACCCTGGAAGGTGATGTTTGTGGTGTGGAGCCTCCTGG
    lLysGlyThrLeuGluGlyAspValCysGlyValGluProProGl
361 CCCAGGAGACACAGTAGTCCAGGGAGACCTGCAGGAGACCACCGT
    yProGlyAspThrValValGlnGlyAspLeuGlnGluThrThrVa
406 GGTGACAGGCCTGGGACCAGACACACAGGACCTGGAAGGCCAGAG
   lValThrGlyLeuGlyProAspThrGlnAspLeuGluGlyGlnSe
451 CCCTCCACAGAGCCTGCCTTCAACCCCCAAAGCAGCTTGGATCAG
    rProProGlnSerLeuProSerThrProLysAlaAlaTrpIleAr
496 GGAGGAGGGCCGCTGCTCCAGCAGTGACGATGACACCGACGTGGA
    gGluGluGlyArgCysSerSerSerAspAspAspThrAspValAs
541 CATGGAGGGTCTGCGGAGACGGCGGGGCCGGGAGGCCGGCCCACC
    pMetGluGlyLeuArgArgArgArgGlyArgGluAlaGlyProPr
586 TCAGCCCATGGTGCCCCTGGCTGTGGAGAACCAGGCTGGGGGTGA
    oGlnProMetValProLeuAlaValGluAsnGlnAlaGlyGlyGl
631 GGGTGCAGGCGGGGAGCTGGGCATCTCCCTCAACATGTGCCTCCT
    uGlyAlaGlyGlyGluLeuGlyIleSerLeuAsnMetCysLeuLe
676 TGGGGCCCTGGGTCTGCTTGGCCTGGGGGTCCTCCTCTTCTCAGG
    uGlyAlaLeuGlyLeuLeuGlyLeuGlyValLeuLeuPheSerGl
721 TGGCCTCTCAGAGTCTGAGACTGGGCCCATGGAGGAAGTGGAGCG
    yGlyLeuSerGluSerGluThrGlyProMetGluGluValGluAr
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766 GCAGGTCCTCCCAGACCCCGAGGTGCTGGAAGCTGTGGGGGACAG

gGlnValLeuProAspProGluValLeuGluAlaValGlyAspAr

811 GCAGGATGGGCTAAGGGAACAGCTGCAGGCCCCAGTGCCTCCTGA

gGlnAspGlyLeuArgGluGlnLeuGlnAlaProValProProAs

856 CAGTGTCCCCAGCCTGCAAAACATGGGTCTTCTGCTGGACAAGCT

pSerValProSerLeuGlnAsnMetGlyLeuLeuLeuAspLysLe

901 GGCCAAGGAGAACCAGGACATCCGGCTGCTGCAGGCCCAGCTGCA

uAlaLysGluAsnGlnAspIleArgLeuLeuGlnAlaGlnLeuGl

946 GGCCCAAAAGGAAGAGCTTCAGAGCCTGATGCACCAGCCCAAAGG

nAlaGlnLysGluGluLeuGlnSerLeuMetHisGlnProLysGl

991 GCTAGAGGAGGAGAATGCCCAGCTCCGGGGGGCTCTGCAGCAGGG

yLeuGluGluGluAsnAlaGlnLeuArgGlyAlaLeuGlnGlnGl

1036 CGAAGCCTTCCAGCGGGCTCTGGAGTCAGAGCTGCAGCAGCTGCG

yGluAlaPheGlnArgAlaLeuGluSerGluLeuGlnGlnLeuAr

1081 GGCCCGGCTCCAGGGGCTGGAGGCCGACTGTGTCCGGGGCCCAGA

gAlaArgLeuGlnGlyLeuGluAlaAspCysValArgGlyProAs

1126 TGGGGATGGCATCTTCCGTCATGACCGCCTCCGCTTCCGGGATTT

pGlyAspGlyIlePheArgHisAspArgLeuArgPheArgAspPh

1171 TGTGGATGCCCTGGAGGACAGCTTGGAGGAGGTGGCTGTGCAACA

eValAspAlaLeuGluAspSerLeuGluGluValAlaValGlnGl

1216 GACAGGTGATGATGATGAAGTAGATGACTTTGAGGACTTCATCTT

nThrGlyAspAspAspGluValAspAspPheGluAspPheIlePh

1261 CAGCCACTTCTTTGGAGACAAAGCACTGAAGAAGAGGTCAGGGAA

eSerHisPhePheGlyAspLysAlaLeuLysLysArgSerGlyLy

1306 GAAGGACAAGCACTCACAGAGCCCAAGAGCTGCGGGGCCCAGGGA

sLysAspLysHisSerGlnSerProArgAlaAlaGlyProArgGl

1351 GGGGCACAGCCATAGCCACCACCACCACCACCGGGGCTGACACCC

uGlyHisSerHisSerHisHisHisHisHisArgGly (SEQ ID NO:10)

1396 TGCCCCACAGGGAATGGCCTTGGCCTGGCCCAGCCCAAGATCCCA

1441 GCGTTATCTAACTCCTGGAGGGTGGACTCTGTCCTGGCTTGTTTG

1486 GTGTCCTCAGATATCTTTCACACAGTAGAGCAAAATCACCAGCCC

1531 TGCACTGATGTCACTTTATGTAGAAAAAGGCCTTAGCTGGACCTG

1576 CGTTGCCGTCTATGCAAATGCATGCAAATACTCCAGGCCCTGGGA

1621 TGTGGGCTTGTGTTTTGTCACTGTGAAGGGGGAGATGGGAGAGGA (SEQ ID NO:9)

6. MBSP6. CLONE 3207791.0.128, A NOVEL SECRETED PROTEIN

An MBSP6 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of clone 3207791.0.128.

A polynucleotide of the present invention has been identified as clone 3207791.0.128. 3207791.0.128 is a full-length clone of 2739 nucleotides, including the entire coding sequence of a protein from nucleotides 670 to 2275 (also referred to herein as "3207791.0.128 protein").

3207791.0.128 and 3207791.0.59 are likely splice variants. Nucleotides 4 – 1133; 2017 - 2552 of 3207791.0.128 and nucleotides 1 – 1108; 1129 - 1665 of 3207791.0.59 are 99.88% identical; amino acid acids 1-155 and 450-535 of 3207791.0.128 and nucleotides 197 – 351 and 352-437 of 3207791.0.59 are 99.585% identical.

The nucleotide sequence of 3207791.0.128 is reported in SEQ ID NO:11. The predicted amino acid sequence of the 3207791.0.128 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:12.

The nucleotide sequence disclosed herein for 3207791.0.128 was searched against the GenBank databases using BLASTN search protocols. The BLASTN search showed 98% homology (1029 of 1042 nucleotides) to the human clone A9A2BRB7 (CAC)n/(GTG)n repeat-containing mRNA, 1047 bp (GenBank-Accession Number U00952). The amino acid sequence of 535 amino acids did not show any significant homologies to known proteins

The nucleotide sequence and amino acid sequence disclosed herein for 3207791.0.128 was searched against other databases using SignalPep and PSort search protocols. 3207791.0.128 seems to have a cleavable amino terminal signal peptide with a cleavage site most likely between position 22 and 23 (GLS-ES). The protein is most likely located outside

of the cell. The predicted molecular weight is 60290.7 daltons. 3207791.0.128 is most likely a secreted protein.

The 3207791.0.128 nucleic acid and encoded polypeptide have the following sequences:

```
1 CTGCAACAAGTTAAGCTGAAGACCGAAGCAAGAGCTGGTTCAGGT
46 GGCAGCCACAGCAGCCTCAGGGACCTCAGCAACTATGGCCTCCTG
91 CCCAGACTCTGATAATAGCTGGGTGCTTGCTGGCTCCGAGAGCCT
136 GCCAGTGGAGACACTGGGCCCGGCATCCAGGATGGACCCAGAATC
181 TGAGAGAGCCCTGCAGGCCCCTCACAGCCCCTCCAAGACAGATGG
226 GAAAGAATTAGCTGGGACCATGGATGGAGAAGGGACGCTCTTCCA
271 GACTGAAAGCCCTCAGTCTGGCAGCATTCTAACAGAGGAGACTGA
316 GGTCAAGGGCACCCTGGAAGGTGATGTTTGTGGTGTGGAGCCTCC
361 TGGCCCAGGAGACACAGTAGTCCAGGGAGACCTGCAGGAGACCAC
406 CGTGGTGACAGGCCTGGGACCAGACACACAGGACCTGGAAGGCCA
451 GAGCCCTCCACAGAGCCTGCCTTCAACCCCCAAAGCAGCTTGGAT
496 CAGGGAGGAGGGCCGCTGCTCCAGCAGTGACGATGACACCGACGT
541 GGACATGGAGGGTCTGCGGAGACGGCGGGGCCGGGAGGCCGGCCC
586 ACCTCAGCCCATGGTGCCCCTGGCTGTGGAGAACCAGGCTGGGGG
631 TGAGGGTGCAGGCGGGGAGCTGGGCATTTTCCCCTCAACATGTGC
                                                MetCys
676 CTCCTTGGGGCCCTGGTTCTGCTTGGCCTGGGGGTCCTCCTCTTC
    LeuLeuGlyAlaLeuValLeuLeuGlyLeuGlyValLeuLeuPhe
721 TCAGGTGGCCTCTCAGAGTCTGAGACTGGGCCCATGGAGGAAGTG
    SerGlyGlyLeuSerGluSerGluThrGlyProMetGluGluVal
766 GAGCGGCAGGTCCTCCCAGACCCCGAGGTGCTGGAAGCTGTGGGG
    GluArgGlnValLeuProAspProGluValLeuGluAlaValGly
811 GACAGGCAGGATGGGCTAAGGGAACAGCTGCAGGCCCCAGTGCCT
    AspArgGlnAspGlyLeuArgGluGlnLeuGlnAlaProValPro
856 CCTGACAGTGTCCCCAGCCTGCAAAACATGGGTCTTCTGCTGGAC
    ProAspSerValProSerLeuGlnAsnMetGlyLeuLeuLeuAsp
```

901 AAGCTGGCCAAGGAGAACCAGGACATCCGGCTGCTGCAGGCCCAG

LysLeuAlaLysGluAsnGlnAspIleArgLeuLeuGlnAlaGln

946 CTGCAGGCCCAAAAGGAAGAGCTTCAGAGCCTGATGCACCAGCCC

LeuGlnAlaGlnLysGluGluLeuGlnSerLeuMetHisGlnPro

991 AAAGGGCTAGAGGAGGAGAATGCCCAGCTCCGGGGGGCTCTGCAG

LysGlyLeuGluGluGluAsnAlaGlnLeuArgGlyAlaLeuGln

1036 CAGGGCGAAGCCTTCCAGCGGGCTCTGGAGTCAGAGCTGCAGCAG

GlnGlyGluAlaPheGlnArgAlaLeuGluSerGluLeuGlnGln

1081 CTGCGGGCCCGGCTCCAGGGGCTGGAGGCCGACTGTGTCCGGGGC

LeuArgAlaArgLeuGlnGlyLeuGluAlaAspCysValArgGly

1126 CCAGATGGGGTGTGCCTCAGTGGGGGTAGAGGCCCACAGGGTGAC

ProAspGlyValCysLeuSerGlyGlyArgGlyProGlnGlyAsp

1171 AAGGCCATCAGGGAGCAAGGCCCCAGGGAGCAGGAGCCAGAACTC

LysAlaIleArgGluGlnGlyProArgGluGlnGluProGluLeu

1216 AGCTTCCTGAAGCAGAAGGAACAGCTGGAGGCTGAGGCACAGGCA

SerPheLeuLysGlnLysGluGlnLeuGluAlaGluAlaGlnAla

1261 TTAAGGCAAGAGTTAGAGAGGCAGCGACGGCTGCTGGGGTCTGTA

LeuArgGlnGluLeuGluArgGlnArgArgLeuLeuGlySerVal

1306 CAGCAGGATCTGGAGAGGAGCTTGCAGGATGCCAGCCGCGGGGAC

GlnGlnAspLeuGluArgSerLeuGlnAspAlaSerArgGlyAsp

1351 CCAGCTCATGCTGGCTTGGCTGAGCTGGGCCACAGATTGGCCCAG

ProAlaHisAlaGlyLeuAlaGluLeuGlyHisArgLeuAlaGln

1396 AAACTGCAGGGCCTGGAGAACTGGGGCCAGGACCCTGGGGTCTCT

LysLeuGlnGlyLeuGluAsnTrpGlyGlnAspProGlyValSer

1441 GCCAATGCCTCAAAGGCCTGGCACCAGAAGTCCCACTTCCAGAAT

AlaAsnAlaSerLysAlaTrpHisGlnLysSerHisPheGlnAsn

1486 TCTAGGGAGTGGAGTGGAAAGGAAAAGTGGTGGGATGGGCAGAGA

SerArgGluTrpSerGlyLysGluLysTrpTrpAspGlyGlnArg

1531 GACCGGAAGGCTGAGCACTGGAAACATAAGAAGGAAGAATCTGGC

AspArgLysAlaGluHisTrpLysHisLysLysGluGluSerGly

1576 CGGGAAAGGAAGAAGAACTGGGGAGGTCAGGAGGACAGGGAGCCA

ArgGluArgLysLysAsnTrpGlyGlyGlnGluAspArgGluPro

1621 GCAGGAAGGTGGAAGGAGGGCAGGCCAAGGGTGGAGGAGTCGGGG

AlaGlyArgTrpLysGluGlyArgProArgValGluGluSerGly

1666 AGCAAGAAGGAGGGCAAGCGACAGGGCCCGAAGGAACCCCCAAGG

SerLysLysGluGlyLysArgGlnGlyProLysGluProProArg

1711 AAAAGTGGTAGCTTCCACTCCTCTGGAGAAAAGCAGAAGCAACCT

LysSerGlySerPheHisSerSerGlyGluLysGlnLysGlnPro

1756 CGGTGGAGGGAAGGGACTAAGGGCAGCCATGACCCCCTGCCATCC

ArgTrpArgGluGlyThrLysGlySerHisAspProLeuProSer

1801 TGGGCAGAGCTGTTGAGGCCCAAGTACCGGGCACCCCAGGGCTGC

TrpAlaGluLeuLeuArgProLysTyrArgAlaProGlnGlyCys

1846 TCAGGTGTGGACGAGTGTGCCCGGCAGGAGGGCCTGACTTTCTTT

SerGlyValAspGluCysAlaArgGlnGluGlyLeuThrPhePhe

1891 GGCACAGAGCTAGCCCCAGTGCGGCAACAGGAGCTGGCCTCTCTG

GlyThrGluLeuAlaProValArgGlnGlnGluLeuAlaSerLeu

1936 CTAAGAACATACTTGGCACGGCTGCCCTGGGCTGGGCAGCTGACC

LeuArgThrTyrLeuAlaArgLeuProTrpAlaGlyGlnLeuThr

1981 AAGGAGCTACCCCTCTCACCTGCTTTCTTTGGTGAGGATGGCATC

LysGluLeuProLeuSerProAlaPhePheGlyGluAspGlyIle

2026 TTCCGTCATGACCGCCTCCGCTTCCGGGATTTTGTGGATGCCCTG

PheArgHisAspArgLeuArgPheArgAspPheValAspAlaLeu

2071 GAGGACAGCTTGGAGGAGGTGGCTGTGCAACAGACAGGTGATGAT

GluAspSerLeuGluGluValAlaValGlnGlnThrGlyAspAsp

2116 GATGAAGTAGATGACTTTGAGGACTTCATCTTCAGCCACTTCTTT

AspGluValAspAspPheGluAspPheIlePheSerHisPhePhe

2161 GGAGACAAAGCACTGAAGAAGAGGTCAGGGAAGAAGGACAAGCAC

GlyAspLysAlaLeuLysLysArgSerGlyLysLysAspLysHis

2206 TCACAGAGCCCAAGAGCTGCGGGGCCCAGGGAGGGGCACAGCCAT

SerGlnSerProArgAlaAlaGlyProArgGluGlyHisSerHis

2251 AGCCACCACCACCACCACCGGGGCTGACACCCTGCCCCACAGGGA

SerHisHisHisHisHisArgGly (SEQ ID NO:12)

2296 ATGGCCTTGGCCTGGCCCAGCCCAAGATCCCAGCGTTATCTAACT

2341 CCTGGAGGGTGGACTCTGTCCTGGCTTGTTTGGTGTCCTCAGATA

2386 TCTTTCACACAGTAGAGCAAAATCACCAGCCCTGCACTGATGTCA

2431 CTTTATGTAGAAAAAGGCCTTAGCTGGACCTGCGTTGCCGTCTAT

2476 GCAAATGCATGCAAATACTCCAGGCCCTGGGATGTGGGCTTGTGT

2521 TTTGTCACTGTGAAGGGGGAGATGGGAGAGGAGCCTGTTTTGGGG

2566 TGGGGTCTGGGGAAGGCAATCTGATTCTGAAGCTAAAGAGCTTTC

2611 ATCCTCTTGAGTGTATGTCCCCATAGTGGGCCCCTTGACCCACAT

2656 GCTGACCGGTGCCTTGGGATTTGACTAGAGTTGCTGCTTCCAAAC

2701 CTAACAAAACCCCAGGGTAAGTCCTCGTGCTGGGCCTCG (SEQ ID NO:11)

7. MBSP7. CLONE 3499605.0.64, HOMOLOG TO TUMOR SUPPRESSOR GENE N33

An MBSP7 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of clone 3499605.0.64.

A polynucleotide of the present invention has been identified as clone 3499605.0.64. 3499605.0.64 is a full-length clone of 681 nucleotides, including the entire coding sequence of a protein from nucleotides 43 to 463 (also referred to herein as "3499605.0.64 protein"). The clone was originally obtained from pituitary gland tissues.

The nucleotide sequence of 3499605.0.64 is reported in SEQ ID NO:13. The predicted amino acid sequence of the 3499605.0.64 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:14.

Searches in publicly available GenBank database BLASTP showed that the amino acid sequence of 140 amino acids is 75% identical (100/132) to the sequence of rat implantation-association protein (335 aa) (ACC:O35777) and 72% homolog (89/122) to the sequence of human N33 protein (348 aa) (ACC: Q13454). N33 is a candidate tumor suppressor gene located on chromosome 8p22 and was found to be silenced by a methylation mechanism in most colon cancer cell lines and some primary colorectal tumours (Levy et al., Genes

Chromosomes Cancer 1999 24(1):42-7; MacGrogan et al., 1996, Genomics 35 (1), 55-65). Based upon homology, 3499605.0.64 proteins and each homologous protein or peptide may share at least some activity.

The nucleotide sequence disclosed herein for 3499605.0.64 was searched against the GenBank databases using BLASTN search protocols. The BLASTN search showed 84% identities (350 of 413 nucleotides) to the mRNA encoding rat implantation-associated protein (IAG2) (1219 bp) (GenBank-Accession Number AF008554) and 71% identities (221 of 311 nucleotides) to the mRNA encoding human N33 protein (1342 bp) (GenBank-Accession Number U42349).

The nucleotide sequence and amino acid sequence disclosed herein for 3499605.0.64 was searched against other databases using SignalPep and PSort search protocols. 3499605.0.64 seems to have a cleavable amino terminal signal peptide with a cleavage site between positions 29 and 30 (ASA-QR). The protein is most likely located outside of the cell. The predicted molecular weight is 16035.8 daltons.

The 3499605.0.64 nucleic acid and encoded polypeptide have the following sequences:

```
1 CACGCGAGCAAAGTGGCACTTATAGAAGGGAGAGGAGCGAACATG

                                                Met

46 GCAGCGCGTTGGCGGTTTTGGTGTGTCTCTGTGACCATGGTGGTG

   AlaAlaArgTrpArgPheTrpCysValSerValThrMetValVal

91 GCGCTGCTCATCGTTTGCGACGTTCCCTCAGCCTCTGCCCAAAGA

   AlaLeuLeuIleValCysAspValProSerAlaSerAlaGlnArg

136 AAGAAGGAGATGGTGTTATCTGAAAAGGTTAGTCAGCTGATGGAA

    LysLysGluMetValLeuSerGluLysValSerGlnLeuMetGlu

181 TGGACTAACAAAAGACCTGTAATAAGAATGAATGGAGACAAGTTC

    TrpThrAsnLysArgProValIleArgMetAsnGlyAspLysPhe

226 CGTCGCCTTGTGAAAGCCCCACCGAGAAATTACTCCGTTATCGTC

    ArgArgLeuValLysAlaProProArgAsnTyrSerValIleVal

271 ATGTTCACTGCTCTCCAACTGCATAGACAGTGTGTCGTTTGCAAG

    MetPheThrAlaLeuGlnLeuHisArgGlnCysValValCysLys

316 CAAGCTGATGAAGAATTCCAGATCCTGGGCAAACTCCTGGGCGAT
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    GlnAlaAspGluGluPheGlnIleLeuGlyLysLeuLeuGlyAsp
361 ACTCCAGTGCATTCACCAACAGGATATTTTTTTGCCATGGTGGAT
    ThrProValHisSerProThrGlyTyrPhePheAlaMetValAsp
406 TTTGATGAAGGCTCTGATGTATTTCAGATGGTAAGATCTTTGGTT
    PheAspGluGlySerAspValPheGlnMetValArgSerLeuVal
451 TATTCTGCTATATAAAAATAGGTAAAATATAGTGAAAGTGGAATA
    TyrSerAlaIle  (SEQ ID NO:14)
496 TATTTTTTCAGTGGCTATAAGACATTAAATGGGATTGCTCTTATG
541 TAAGATCTAGAGTAGCAAACTAAAAGTTAAACTTGAGCTAACCAT
586 AAAATGTAGTTATCAATGCTAACCTACTAGAATATGGAAATTGAG
631 TAGTATTTAAATAACATACATTTTTATTGTAGTAAAATATATAAA
676 ACAAAC  (SEQ ID NO:13)
```

8. MBSP8. CLONE AQ013000.0.21, A NOVEL KERATINOCYTE GROWTH FACTOR

An MBSP8 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of clone AQ013000.0.21.

A polynucleotide of the present invention has been identified as clone AQ013000.0.21. AQ013000.0.21 is a full-length clone of 670 nucleotides, including the entire coding sequence of a protein from nucleotides 180 to 471 (also referred to herein as "AQ013000.0.21 protein"). The original tissue source of this clone is unknown.

The nucleotide sequence of AQ013000.0.21 is reported in SEQ ID NO:15. The predicted amino acid sequence of the AQ013000.0.21 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:16.

The nucleotide sequence disclosed herein for AQ013000.0.21 was searched against the GenBank databases using BLASTN search protocols. The BLASTN search showed 94% homology (481 of 509 nucleotides) to human keratinocyte growth factor mRNA, 3853 bp (GenBank-Accession Number M60828); the 5' region of AQ013000.0.21 is different.

The amino acid sequence of 97 amino acids is 89.691% % identical to the sequence of

a human keratinocyte growth factor analogue C(1,15,102)S protein of 164 amino acids (patp:W61426, Patent application WO9824813, Amgen Inc). Further searches in publicly available GenBank database BLASTP showed 95% identity (93 of 97 amino acids) with human keratinocyte growth factor precursor (KGF; fibroblast growth factor-7; FGF-7; 194 aa) (SWISSPROT-ACC:P21781).

Keratinocyte growth factor analogues can be administered to a patient to increase the cytoprotection, proliferation and/or differentiation of epithelial cells in the gastrointestinal (GI) tract. The method can be used to treat or prevent gastric and duodenal ulcers, gut toxicity such as in radiation- and chemotherapy-treatment regions, erosions of the GI tract including erosive gastritis, oesophagitis, eophageal reflux or inflammatory bowel diseases such as Crohn's disease or ulcerative colitis. Based upon homology, AQ013000.0.21 proteins and each homologous protein or peptide may share at least some activity.

The nucleotide sequence and amino acid sequence disclosed herein for AQ013000.0.21 was searched against other databases using SignalPep and PSort search protocols. AQ013000.0.21 seems to have an uncleavable amino terminal signal peptide. The protein is most likely located in the membrane of the endoplasmic reticulum. The predicted molecular weight is 11009.6 daltons.

The AQ013000.0.21 nucleic acid and encoded polypeptide have the following sequences:

```
1 CTATTCTGGTGTTTGTGTGTATCTGTTGTGGGTCGGGGTCTTGGG

46 CTGAGTAAATAATCACATTAAAAATTTTAAAAACTTCCGATTAAA

91 ACAGAAATAAGAACAAACGGCCATTCGTGGATCATTTGCAAAGCT

136 GAACGAATACTTGACATGTCTTTCTGTGATTCCTTGCAGATATCA

                                                  M

181 TGGAAATCAGGACAGTAGCAGTTGGGATTGTGGCAATCAAAGGGG

    etGluIleArgThrValAlaValGlyIleValAlaIleLysGlyV

226 TGGAAAGTGAATTCTATCTTGCAACGAACGAGGAAGGAAAACTCT

    alGluSerGluPheTyrLeuAlaThrAsnGluGluGlyLysLeuT

271 ATGCAAAGAAGGAATACAATGAAGATTGTAACTTCAAAGATCTAA

    yrAlaLysLysGluTyrAsnGluAspCysAsnPheLysAspLeuI
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316 TTCTGGAAAACCATTACAACACATATGCAGCAGCTAAATGGACAA
    leLeuGluAsnHisTyrAsnThrTyrAlaAlaAlaLysTrpThrA
361 ACAACGGAGGGGAAATGTTTGTGGCCTTAAATCAAAAGGGGATTC
    snAsnGlyGlyGluMetPheValAlaLeuAsnGlnLysGlyIleP
406 CTGTAAGAGGAAAAAAAAAAAGGAAGGACCAAAAACCAGCCCACT
    roValArgGlyLysLysLysArgLysAspGlnLysProAlaHisP
451 TTCTTCCTATGGCAATAACTTAATTGCATATGGTATATAAAGAAC
    heLeuProMetAlaIleThr  (SEQ ID NO:16)
496 CAGTTCCAGCAGGGAGATTTCTTTAAGTGGACTGTTTTCTTTCTT
541 CTCAAAATTTTCTTTCCTTTTATTTTCTAGAAATCAAGAAAGGCT
586 GGAAAACTACTGAAAAACTGATCAAGCTGGACTTGCGCATTTATG
631 TTTGTTTTAAGGCACTGCATTAAAGAGACATTTGAAAAGT  (SEQ ID NO:15)
```

9. MBSP9. CLONE 16401346.0.337, A NOVEL SECRETED PROTEIN

An MBSP9 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of clone 16401346.0.337.

A polynucleotide of the present invention has been identified as clone 16401346.0.337. 16401346.0.337 is a full-length clone of 1135 nucleotides, including the entire coding sequence of a protein from nucleotides 261 to 980 (also referred to herein as "16401346.0.337 protein"). The protein associated with cura_75_16401346.0.337 is encoded in a negative reading frame (Frame: -3). The sequence shown has been reverse-complemented and renumbered to allow reading of the protein in the expected N to C direction.

The nucleotide sequence of 16401346.0.337 is reported in SEQ ID NO:17. The predicted amino acid sequence of the 16401346.0.337 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:18.

The nucleotide sequence disclosed herein for 16401346.0.337 was searched against the GenBank databases using BLASTN search protocols. The BLASTN search showed 57% homology (194 of 335 nucleotides) to *Geodia cydonium* mRNA for SRCR domain protein-membrane form -isotype 2, 6323 bp (GenBank-Accession Number Y14953).

The amino acid sequence of 240 amino acids was searched in publicly available GenBank database BLASTP and showed no significant homologies to published proteins.

The nucleotide sequence and amino acid sequence disclosed herein for 16401346.0.337 was searched against other databases using SignalPep and PSort search protocols. 16401346.0.337 seems to have a cleavable amino terminal with a signal peptide at position 57 and 58: ILG-KN. The protein is most likely located in the lumen of the lysosome. The predicted molecular weight is 25397.2 daltons.

The 16401346.0.337 nucleic acid and encoded polypeptide have the following sequences:

```
1 TTTGCCTATTTCATTAGCAGATGAGGATCCTCTTGCTGCTCCTAG

46 GCAGGGCCCCACAGAAAACCTCCTCCCAAGTTGCCAGATTGATCC

91 TAGGGGTTTGCACACCCTTTTGTCCTCCTCTTCCCTGCCAGTGAT

136 CTGGGAGTCAGGGGAGGAGGTGAGTTTGCTGAACTTACATGGCCA

181 TCTAAAGCCCAGTGATCATCTTTGAACTTATTCATAAAGATCTCC

226 ACAGGCCCTGTAATCTGGTACACCTGGAGTAGGAGATGAAAATCT

                                        MetLysIleP

271 TCTTTCTTGTAAGTTCCAGCCAAGTGCAGCTCCACGCCACTGTGG

    hePheLeuValSerSerSerGlnValGlnLeuHisAlaThrValV

316 TCGATCATGGTGGCATTGACCTTGCTGTTGGCAACTTGGAAGCCA

    alAspHisGlyGlyIleAspLeuAlaValGlyAsnLeuGluAlaS

361 GTCACTCTGAGCATGGCTGGCTGCTTCTTCCCCTGGTATTCATAG

    erHisSerGluHisGlyTrpLeuLeuLeuProLeuValPheIleG

406 GCCCCTTTCCACAGGGAATTCTTGGCAAAAACATCCCCAGGAATC

    lyProPheProGlnGlyIleLeuGlyLysAsnIleProArgAsnG

451 AAGGCTTGGGTGAGCGGTGGCTCCCGGGCAGTGTTGATGGGTCTC

    lnGlyLeuGlyGluArgTrpLeuProGlySerValAspGlySerP

496 CCACTGGGCCGGACCTCCAGGCAGATGGGCGGCTTGCCTGTCCAG

    roThrGlyProAspLeuGlnAlaAspGlyArgLeuAlaCysProA

541 CTGCCATCCGCCTTGCAGGTGCGGTGCTCGGAGCCACCCTTGAGG

    laAlaIleArgLeuAlaGlyAlaValLeuGlyAlaThrLeuGluG
```

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586 GAGAAGCCCTCCTGGCAGGAGTAGATGAGCGTGTAGCCCATGGAG
    lyGluAlaLeuLeuAlaGlyValAspGluArgValAlaHisGlyG
631 GGCAAATCCAGGGCCCCGACGTTGGCATGCGTTGGCGTCTCTGGC
    lyGlnIleGlnGlyProAspValGlyMetArgTrpArgLeuTrpL
676 TGCCTGCAGTGGTGGGGGACACAGTCAGGTGGGGTTCCACTCCAG
    euProAlaValValGlyAspThrValArgTrpGlySerThrProG
721 GTCAGGTTTGGGAGGCAGGTCCTGGTGGTGGAGCCCTGAAGCAGG
    lyGlnValTrpGluAlaGlyProGlyGlyGlyAlaLeuLysGlnV
766 TAGCCTTTTTGACAACGGAAGAGGACTGTGCTTCCAACCTGGTAG
    alAlaPheLeuThrThrGluGluAspCysAlaSerAsnLeuValA
811 CCCTGAGAATTGTTCTGTATCCCAAACTGTGGCACACCAGGGTCC
    laLeuArgIleValLeuTyrProLysLeuTrpHisThrArgValA
856 GCACACGTGGTCAGGGTCGGATCTATGCAGCTGGGCTGGGTGCCA
    rgThrArgGlyGlnGlyArgIleTyrAlaAlaGlyLeuGlyAlaT
901 CTCCATGTCCCATCATGCTTGGGTCGTTCATGAATTCCATGAGAA
    hrProCysProIleMetLeuGlySerPheMetAsnSerMetArgI
946 TTGTTGGGAAGTTCATGAGTTTAGGAATCAAAGTATAGCATTTAG
    leValGlyLysPheMetSerLeuGlyIleLysVal  (SEQ ID NO:18)
991 GGCCACTTTCTCTGTCCTCGGGAAGGCCCCCACAAGCTTAAGCCC
1036 CTGTATACGTGTGACATGCTGGCCTCAAGATTCATGTCACATATT
1081 GAAATACCAAATAGAAATGTGCAGGAGCGGAGGGACAGCGACACC
1126 TGGTTCTTAA  (SEQ ID NO:17)
```

The protein associated with cura_75_16401346.0.337 is encoded in a negative reading frame. The sequence shown above has been reverse-complemented and renumbered to allow reading of the protein in the expected N to C direction.

10. MBSP10. CLONE 20604798.0.1

An MBSP10 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of clone 20604798.0.1.

A polynucleotide of the present invention has been identified as clone 20604798.0.1.

20604798.0.1 is a full-length clone of 2437 nucleotides, including the entire coding sequence of a secreted protein from nucleotides 147 to 1595 (also referred to herein as "20604798.0.1 protein").

The nucleotide sequence of 20604798.0.1 is reported in SEQ ID NO:19. The predicted amino acid sequence of the 20604798.0.1 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:20.

The nucleotide sequence disclosed herein for 20604798.0.1 was searched against the GenBank databases using BLASTN search protocols. The BLASTN search showed 99% homology (1759 of 1760 nucleotides) to *Homo sapiens* clone 24936 mRNA sequence, 1783 bp (GenBank-Accession Number AF131849). The first 678 nucleotides at the 5' terminus of 20604798.0.1 do not show identities to the 24936 mRNA sequence.

The amino acid sequence of 483 amino acids is 100% identitical of the carboxyl-terminal 182 amino acids to a human "UNKNOWN" protein fragment, 182 aa (TREMBLNEW-ACC:AAD20029). The amino terminal 181 amino acids of 20604798.0.1 did not show significant homologies to known proteins.

The nucleotide sequence and amino acid sequence disclosed herein for 20604798.0.1 was searched against other databases using SignalPep and PSort search protocols. 20604798.0.1 seems to have a cleavable amino terminal signal peptide with a most likely cleavage site between pos. 33 and 34: GRA-LP. The protein is most likely located outside of the cell. The predicted molecular weight is 54857.8 daltons.

The 20604798.0.1 nucleic acid and encoded polypeptide have the following sequences:

```
1 TCCGGAAGGAGACGTGGCGGCGGTTGGGCCGGTGATACCCGGGCG
46 CTTTATAGTCCCGCCGCCTCCTCCTCCACCTCCTCCTCCTCCTCC
91 TCTCCTCCTGGAGCAGAGGAGGTTGTGGCGGTGGCTGGAGAAAGC
136 GGCGGCGGAGGATGGAGGAAGGAGGCGGCGGCGTACGGAGTCTGG
                MetGluGluGlyGlyGlyGlyValArgSerLeuV
181 TCCCGGGCGGGCCGGTGTTACTGGTCCTCTGCGGCCTCCTGGAGG
    alProGlyGlyProValLeuLeuValLeuCysGlyLeuLeuGluA
226 CGTCCGGCGGCGGCCGAGCCCTTCCTCAACTCAGCGATGACATCC
```

laSerGlyGlyGlyArgAlaLeuProGlnLeuSerAspAspIleP

271 CTTTCCGAGTCAACTGGCCCGGCACCGAGTTCTCTCTGCCCACAA

roPheArgValAsnTrpProGlyThrGluPheSerLeuProThrT

316 CTGGAGTTTTATATAAAGAAGATAATTATGTCATCATGACAACTG

hrGlyValLeuTyrLysGluAspAsnTyrValIleMetThrThrA

361 CACATAAAGAAAAATATAAATGCATACTTCCCCTTGTGACAAGTG

laHisLysGluLysTyrLysCysIleLeuProLeuValThrSerG

406 GGGATGAGGAAGAAGAAAAGGATTATAAAGGCCCTAATCCAAGAG

lyAspGluGluGluGluLysAspTyrLysGlyProAsnProArgG

451 AGCTTTTGGAGCCACTATTTAAACAAAGCAGTTGTTCCTACAGAA

luLeuLeuGluProLeuPheLysGlnSerSerCysSerTyrArgI

496 TTGAGTCTTATTGGACTTACGAAGTATGTCATGGAAAACACATTC

leGluSerTyrTrpThrTyrGluValCysHisGlyLysHisIleA

541 GGCAGTACCATGAAGAGAAAGAAACTGGTCAGAAAATAAATATTC

rgGlnTyrHisGluGluLysGluThrGlyGlnLysIleAsnIleH

586 ACGAGTACTACCTTGGGAATATGTTGGCCAAGAACCTTCTATTTG

isGluTyrTyrLeuGlyAsnMetLeuAlaLysAsnLeuLeuPheG

631 AAAAAGAACGAGAAGCAGAAGAAAAGGAAAAATCAAATGAGATTC

luLysGluArgGluAlaGluGluLysGluLysSerAsnGluIleP

676 CCACTAAAAATATCGAAGGTCAGATGACACCATACTATCCTGTGG

roThrLysAsnIleGluGlyGlnMetThrProTyrTyrProValG

721 GAATGGGAAATGGTACACCTTGTAGTTTGAAACAGAACCGGCCCA

lyMetGlyAsnGlyThrProCysSerLeuLysGlnAsnArgProA

766 GATCAAGTACTGTGATGTACATATGTCATCCTGAATCTAAGCATG

rgSerSerThrValMetTyrIleCysHisProGluSerLysHisG

811 AAATTCTTTCAGTAGCTGAAGTTACAACTTGTGAATATGAAGTTG

luIleLeuSerValAlaGluValThrThrCysGluTyrGluValV

856 TCATTTTGACACCACTCTTGTGCAGTCATCCTAAATATAGGTTCA

alIleLeuThrProLeuLeuCysSerHisProLysTyrArgPheA

901 GAGCATCTCCTGTGAATGACATATTTTGTCAATCACTGCCAGGAT

rgAlaSerProValAsnAspIlePheCysGlnSerLeuProGlyS

946 CTCCATTTAAGCCCCTCACCCTGAGGCAGCTGGAGCAGCAGGAAG

erProPheLysProLeuThrLeuArgGlnLeuGluGlnGlnGluG

991 AAATACTAAGGGTGCCTTTTAGGAGAAATAAAGAGGAAGATTTGC

luIleLeuArgValProPheArgArgAsnLysGluGluAspLeuG

1036 AATCAACTAAAGAAGAGAGATTTCCAGCGATCCACAAGTCGATTG

lnSerThrLysGluGluArgPheProAlaIleHisLysSerIleA

1081 CTATTGGCTCTCAGCCAGTGCTCACTGTTGGGACAACCCACATAT

laIleGlySerGlnProValLeuThrValGlyThrThrHisIleS

1126 CCAAATTGACAGATGACCAACTCATAAAAGAGTTTCTTAGTGGTT

erLysLeuThrAspAspGlnLeuIleLysGluPheLeuSerGlyS

1171 CTTACTGCTTTCGTGGGGGTGTCGGTTGGTGGAAATATGAATTCT

erTyrCysPheArgGlyGlyValGlyTrpTrpLysTyrGluPheC

1216 GCTATGGCAAACATGTACATCAATACCATGAGGACAAGGATAGTG

ysTyrGlyLysHisValHisGlnTyrHisGluAspLysAspSerG

1261 GGAAAACCTCTGTGGTTGTCGGGACATGGAACCAAGAAGAGCATA

lyLysThrSerValValValGlyThrTrpAsnGlnGluGluHisI

1306 TTGAATGGGCTAAGAAGAATACTGCTAGAGCTTATCATCTTCAAG

leGluTrpAlaLysLysAsnThrAlaArgAlaTyrHisLeuGlnA

1351 ACGATGGTACCCAGACAGTCAGGATGGTGTCACATTTTTATGGAA

spAspGlyThrGlnThrValArgMetValSerHisPheTyrGlyA

1396 ATGGAGATATTTGTGATATAACTGACAAACCAAGACAGGTGACTG

snGlyAspIleCysAspIleThrAspLysProArgGlnValThrV

1441 TAAAACTAAAGTGCAAAGAATCAGATTCACCTCATGCTGTTACTG

alLysLeuLysCysLysGluSerAspSerProHisAlaValThrV

1486 TATATATGCTAGAGCCTCACTCCTGTCAATATATTCTTGGGGTTG

alTyrMetLeuGluProHisSerCysGlnTyrIleLeuGlyValG

1531 AATCTCCAGTGATCTGTAAAATCTTAGATACAGCAGATGAAAATG

luSerProValIleCysLysIleLeuAspThrAlaAspGluAsnG

1576 GACTTCTTTCTCTCCCCAACTAAAGGATATTAAAGTTAGGGGAAA

lyLeuLeuSerLeuProAsn (SEQ ID NO:20)

1621 GAAAAGATCATTGAAAGTCATGATAATTTCTGTCCCACTGTGTCT

1666 CATTATAGAGTTCTCAGCCATTGGACCTCTTCTAAAGGATGGTAT

1711 AAAATGACTCTCAACCACTTTGTGAATACATATGTGTATATAAGA

1756 GGTTATTGATAAACTTCTGAGGCAGACATTTGTCTCGCTTTTTTT

1801 CATTTTTGTTGTGTCTTATAAACTGACTGTTTTTCTTTGCTTGGA

1846 TACTGTGATTCCAAAATAAATCTCATCCAAGCAAGTTAGAGTCCA

1891 GCCTAATCAAATGTCATAATTGTTGTACCTATTGAAAGTTTTTAA

1936 ATAATAGATTTATTATGTAAATTATAGTATATGTAAGTAGCTAAT

1981 GAAGTAAAGATCATGAAGAAAGAAATTGATAGGTGTAAATGAGAG

2026 ACCATGTAAAATATGTAAATTCTAGTACCTGAAATCCTTTCAACA

2071 GATTTTTATATAGCAACTGCTCTCTGCAAGTAGTTAAACTAGAAA

2116 CTGGGCACATGGTAGAGGCTCACATGGGAGTTGTCCTCACCCTTG

2161 TTAATCTCAAGAAACTCTTATTTATAATAGGTTGCTTCTCTCTCA

2206 GAACTTTTATCTATTACTTTTTTCTTCTTATGAGTATGTTTACTC

2251 TCAGAGTATCTATCTGATGTAGACAGTTGGTGATGCTTCTGAGAC

2296 TCAGAATGGTTTACTCTAACAAAACACTGTGCTGTCTATCCCTTG

2341 TACTTGCCTACTGTAATATGGATTTCACTTCTGAACAGTTTACAG

2386 CACAATATTTATTTTAAAGTGAATAAAATGTCCACAAGCAGTGTT

2431 GTCATGT (SEQ ID NO:19)

11. MBSP11. CLONE 27978313.0.29, A NOVEL MEMBRANE PROTEIN

An MBSP11 nucleic acid and polypeptide according to the invention includes the nucleic acid and encoded polypeptide sequence of clone 27978313.0.29.

A polynucleotide of the present invention has been identified as clone 27978313.0.29. 27978313.0.29 is a full-length clone of 1288 nucleotides, including the entire coding sequence of a protein from nucleotides 733 to 1003 (also referred to herein as "27978313.0.29 protein").

The nucleotide sequence of 27978313.0.29 is reported in SEQ ID NO:21. The predicted amino acid sequence of the 27978313.0.29 protein corresponding to the foregoing

nucleotide sequence is reported in SEQ ID NO:22.

Searches in publicly available GenBank database BLASTP showed that the amino acid sequence of 90 amino acids is 61% homolog (27 of 44 amino acids) to *Rhodospirillum rubrum* nicotinamide nucleotide transhydrogenase, subunit beta (464 amino acid acids) (SPTREMBL-ACC:Q59765). Based upon homology, 27978313.0.29 proteins and each homologous protein or peptide may share at least some activity.

The nucleotide sequence and amino acid sequence disclosed herein for 27978313.0.29 was searched against other databases using SignalPep and PSort search protocols. 27978313.0.29 seems to have a cleavable amino terminal signal peptide with a cleavage site between position 46 and 47 (CWG-AT). The protein is most likely located in the plasma membrane. The predicted molecular weight of the putative membrane protein is 9358.1 daltons.

The 27978313.0.29 nucleic acid and encoded polypeptide have the following sequences:

```
1 TTACTCACTATAGGGCTCGAGCGGCTGCCCGGGCAGGTGAACTGG
46 TGAGCCTGGCTGTGTATCACCTGGATAACCTCTGAGCTGAAGAGC
91 TGTGCCAGCAAAGCCCTTGAGCTCATGTGGGCATGGGGGACAGTC
136 TGAGGACAGGAGGAACAGCAAGGAGACCTCAGGCTGCGCTCAGGA
181 GGCTTCGGTGGCAGGTCAGATTGCTCTTACAATTTGGCAACCATC
226 TTATGAATATTGTGGGTTAAAAGAAATAATCAGTGTCACCGGTAG
271 CCAAGTTTTTAATGTTAAAAAAGATGACAATATAGAATAAAATGA
316 ACCCCTGCAGGGTTCCGTGGGGAGTATTACCATGAGAGCATCATT
361 CTGTGCGTAGGCTGTGCTCCCTGCCAGCGGTGGGCTTCCCAGCAG
406 GCGGCCTTGGCTCTGCGTGTGACATGCTCAGCCTGCAGCACCGTG
451 TGCCCTCCTGAGCAGCTCTGCTCTGGTGGATGCTCTCAGCAGAAA
496 ACACACAACTTCGTGGTCCCCTCTTCTGCCCCTGGGTGGGCCAGG
541 GTGCGGGTGTGCATCCCATGCCCAGGACTGCCAGGCAGGCCGCAT
586 GGTCAGCCACCAGGGTCAGGTGTCAGGCGCTGCTCCAGACGCGTG
631 CGTCTCTGATCTTGGGCATGGCTCAGCAGCCTCCGGCTTGGCCGT
676 GGGTGGTTCCTGCTCACCTGTTTCTGAGTGACCAACCCTGAAACT
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721 TCTGGGCCTAACATGAGGCCTCATCACCTTTCCCGCATCTGTGGG
                MetArgProHisHisLeuSerArgIleCysGly
766 CTGGTGGGTGGGGCTCTGGCCCCACAGGTTATTCCTGCACTGCCA
    LeuValGlyGlyAlaLeuAlaProGlnValIleProAlaLeuPro
811 CCCGTGGTGGCTGGGCTGGCAGCCCTCAGGGGCCTTGCTGTCATG
    ProValValAlaGlyLeuAlaAlaLeuArgGlyLeuAlaValMet
856 GGTGTCTGCTGGGGTGCCACAGTTTCCCCCACATGGATCTCTCCA
    GlyValCysTrpGlyAlaThrValSerProThrTrpIleSerPro
901 GGCAGAGTTTTATATCCTCACCCTAGAGCAGAGTTTTTGGACTTC
    GlyArgValLeuTyrProHisProArgAlaGluPheLeuAspPhe
946 TTGCTCTCAGGACCCCTTAAAGTCCTTGTTGAGGCTGGGCATGGT
    LeuLeuSerGlyProLeuLysValLeuValGluAlaGlyHisGly
991 GGCTTCTGCCTGTAATCCCAGCACTTTGGGAGGCCAAGGTGGGAG
    GlyPheCysLeu  (SEQ ID NO:22)
1036 GATCACTTGAGACTGGGAGTTCAGGACCAGCCTGAGCAACATAGG
1081 GAAACCCCATCTGTACAAAAAAGTAAAAAGAATTAGCCAGATGTG
1126 ATGGTGTATGCCTCTAGTTCCAGTTGTTTGGGAGGCTGAGGCGGG
1171 AGGATTGCTTGAACCCAAGAGGCTGAGGCTGCGGTGAGCTCTGCA
1216 CCGCTGCACTCCAGTCTCAGTGATAGTGAGACCCTGCCTCTAAAA
1261 TTAAAAAAAAAAGTCCTTATTGAGTTTAC  (SEQ ID NO:21)
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Nucleic Acids

One aspect of the invention pertains to isolated nucleic acid molecules that encode MBSPX proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify MBSPX-encoding nucleic acids (*e.g.*, MBSPX mRNA) and fragments for use as PCR primers for the amplification or mutation of MBSPX nucleic acid molecules.

As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA

or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

An "isolated" nucleic acid molecule is one that is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated MBSPX nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

In some embodiments, the MBSPX nucleic acids encode a mature form a MBSPX polypeptide. As used herein, the term a "mature" form of a polypeptide or protein is the product of a naturally occurring polypeptide or precursor form or MBSPX-protein. The naturally occurring polypeptide, precursor or MBSPX-protein includes, by way of non-limiting example, the full length gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or MBSPX-protein encoded by an open reading frame described herein. The product "mature" form arises, again by way of non-limiting example, as a result of one or more naturally occurring processing steps as they may take place within the cell, or host cell, in which the gene product arises. Examples of such processing steps leading to a "mature" form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an open reading frame, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine, would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from

residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Further as used herein, a "mature" form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by way of non-limiting example, glycosylation, myristylation, or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or a combination of any of them.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, or a complement of any of these nucleotide sequences, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequences of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23, as a hybridization probe, MBSPX molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook *et al.*, eds., MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, eds., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to MBSPX nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23. In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 or a portion of this nucleotide sequence. A nucleic acid molecule that is complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 is one that is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9,

11, 13, 15, 17, 19, 21, or 23 that it can hydrogen bond with little or no mismatches to the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23, thereby forming a stable duplex..

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 , *e.g.*, a fragment that can be used as a probe or primer or a fragment encoding a biologically active portion of an MBSPX. Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type. Homologs are nucleic acid sequences or amino acid sequences of a particular gene that are derived from different species.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 30%, 50%, 70%, 80%, or 95% identity (with a preferred identity of 80-95%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below.

The nucleotide sequence determined from the cloning of a human MBSPX gene allows for the generation of probes and primers designed for use in identifying and/or cloning MBSPX homologues in other cell types, *e.g.* from other tissues, as well as MBSPX homologues from other mammals. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 consecutive sense strand nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23, or an anti-sense strand nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23, or of a naturally occurring mutant of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23.

Probes based on the human MBSPX nucleotide sequence can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.* the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress an MBSPX protein, such as by measuring a level of an MBSPX-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting MBSPX mRNA levels or determining whether a genomic MBSPX gene has been mutated or deleted.

"A polypeptide having a biologically active portion of MBSPX" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically active portion of MBSPX" can be prepared by isolating a portion of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 that encodes a polypeptide having a MBSPX biological activity (the biological activities of the MBSPX proteins are described above), expressing the encoded portion of MBSPX protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of MBSPX.

MBSPX variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 due to

degeneracy of the genetic code and thus encode the same MBSPX protein as that encoded by the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 . In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24.

In addition to the human MBSPX nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of MBSPX may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the MBSPX gene may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding an MBSPX protein, preferably a mammalian MBSPX protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the MBSPX gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in MBSPX that are the result of natural allelic variation and that do not alter the functional activity of MBSPX are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding MBSPX proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the MBSPX cDNAs of the invention can be isolated based on their homology to the human MBSPX nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. For example, a soluble human MBSPX cDNA can be isolated based on its homology to human membrane-bound MBSPX cDNA. Likewise, a membrane-bound human MBSPX cDNA can be isolated based on its homology to soluble human MBSPX cDNA.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13,

15, 17, 19, 21, or 23. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250 or 500 nucleotides in length. In another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (*i.e.*, nucleic acids encoding MBSPX proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

Stringent conditions are known to those skilled in the art and can be found in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions are hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C, followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 , or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well-known in the art. See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY,

John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations). See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981, *Proc Natl Acad Sci USA 78*: 6789-6792.

Conservative mutations

In addition to naturally-occurring allelic variants of the MBSPX sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23, thereby leading to changes in the amino acid sequence of the encoded MBSPX protein, without altering the functional ability of the MBSPX protein. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of MBSPX without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the MBSPX proteins of the present invention, are predicted to be particularly unamenable to alteration.

Another aspect of the invention pertains to nucleic acid molecules encoding MBSPX proteins that contain changes in amino acid residues that are not essential for activity. Such MBSPX proteins differ in amino acid sequence from SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18,

20, 22, or 24, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 45% homologous to the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24. Preferably, the protein encoded by the nucleic acid molecule is at least about 60% homologous to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24, more preferably at least about 70% homologous to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24, still more preferably at least about 80% homologous to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24, even more preferably at least about 90% homologous to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24, and most preferably at least about 95% homologous to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24.

An isolated nucleic acid molecule encoding a MBSPX protein homologous to the protein of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in MBSPX is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a MBSPX coding sequence, such as by saturation mutagenesis, and the resultant

mutants can be screened for MBSPX biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

In one embodiment, a mutant MBSPX protein can be assayed for (1) the ability to form protein:protein interactions with other MBSPX proteins, other cell-surface proteins, or biologically active portions thereof, (2) complex formation between a mutant MBSPX protein and a MBSPX ligand; (3) the ability of a mutant MBSPX protein to bind to an intracellular target protein or biologically active portion thereof; (*e.g.* avidin proteins).

Antisense

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire MBSPX coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of an MBSPX protein of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24, or antisense nucleic acids complementary to an MBSPX nucleic acid sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding MBSPX. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding MBSPX. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding MBSPX disclosed herein (*e.g.*, SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of MBSPX mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of MBSPX mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of MBSPX mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used.

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a MBSPX protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res* 15: 6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res* 15: 6131-6148) or a chimeric RNA -DNA analogue (Inoue *et al.* (1987) *FEBS Lett* 215: 327-330).

Ribozymes and PNA moieties

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave MBSPX mRNA transcripts to thereby inhibit translation of MBSPX mRNA. A ribozyme having specificity for an

MBSPX-encoding nucleic acid can be designed based upon the nucleotide sequence of an MBSPX cDNA disclosed herein (*i.e.*, SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a MBSPX-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Pat. No. 4,987,071; and Cech *et al.* U.S. Pat. No. 5,116,742. Alternatively, MBSPX mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, MBSPX gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the MBSPX (*e.g.*, the MBSPX promoter and/or enhancers) to form triple helical structures that prevent transcription of the MBSPX gene in target cells. See generally, Helene. (1991) *Anticancer Drug Des.* 6: 569-84; Helene. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14: 807-15.

In various embodiments, the nucleic acids of MBSPX can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup *et al.* (1996) *Bioorg Med Chem* 4: 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup *et al.* (1996) above; Perry-O'Keefe *et al.* (1996) *PNAS* 93: 14670-675.

PNAs of MBSPX can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of MBSPX can also be used, *e.g.*, in the analysis of single base pair mutations in a gene by, *e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination

with other enzymes, *e.g.*, S1 nucleases (Hyrup B. (1996) above); or as probes or primers for DNA sequence and hybridization (Hyrup *et al.* (1996), above; Perry-O'Keefe (1996), above).

In another embodiment, PNAs of MBSPX can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of MBSPX can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, *e.g.*, RNase H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup (1996) above). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996) above and Finn *et al.* (1996) *Nucl Acids Res* 24: 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA (Mag *et al.* (1989) *Nucl Acid Res* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn *et al.* (1996) above). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. See, Petersen *et al.* (1975) *Bioorg Med Chem Lett* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre *et al.*, 1987, Proc. *Natl. Acad. Sci.* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (See, *e.g.*, Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon, 1988, *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, etc.

MBSPX proteins

One aspect of the invention pertains to isolated MBSPX proteins, and biologically active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-MBSPX antibodies. In one embodiment, native MBSPX proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, MBSPX proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a MBSPX protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the MBSPX protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of MBSPX protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of MBSPX protein having less than about 30% (by dry weight) of non-MBSPX protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-MBSPX protein, still more preferably less than about 10% of non-MBSPX protein, and most preferably less than about 5% non-MBSPX protein. When the MBSPX protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of MBSPX protein in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of MBSPX protein having less than about 30% (by dry weight) of chemical precursors or non-MBSPX chemicals, more preferably less than about 20% chemical precursors or

non-MBSPX chemicals, still more preferably less than about 10% chemical precursors or non-MBSPX chemicals, and most preferably less than about 5% chemical precursors or non-MBSPX chemicals.

Biologically active portions of an MBSPX protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the MBSPX protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24, that include fewer amino acids than the full length MBSPX proteins, and exhibit at least one activity of a MBSPX protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the MBSPX protein. A biologically active portion of an MBSPX protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

Moreover, other biologically active portions, in which other regions of the protein are deleted, can be pepared by recombinant techniques and evaluated for one or more of the functional activities of a native MBSPX protein.

In an embodiment, the MBSPX protein has an amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24. In other embodiments, the MBSPX protein is substantially homologous to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24 and retains the functional activity of the protein of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24 yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail below. Accordingly, in another embodiment, the MBSPX protein is a protein that comprises an amino acid sequence at least about 45% homologous to the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24 and retains the functional activity of the MBSPX proteins of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24.

Determining homology between two or more sequences

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the

corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. See, *Needleman and Wunsch* 1970 *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 .

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison *(i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region.

Chimeric and fusion proteins

The invention also provides MBSPX chimeric or fusion proteins. As used herein, an MBSPX "chimeric protein" or "fusion protein" comprises an MBSPX polypeptide operatively linked to a non-MBSPX polypeptide. An "MBSPX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to MBSPX, whereas a "non-MBSPX

polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the MBSPX protein, *e.g.*, a protein that is different from the MBSPX protein and that is derived from the same or a different organism. Within an MBSPX fusion protein the MBSPX polypeptide can correspond to all or a portion of an MBSPX protein. In one embodiment, an MBSPX fusion protein comprises at least one biologically active portion of an MBSPX protein. In another embodiment, an MBSPX fusion protein comprises at least two biologically active portions of an MBSPX protein. In yet another embodiment, an MBSPX fusion protein comprises at least three biologically active portions of an MBSPX protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the MBSPX polypeptide and the non-MBSPX polypeptide are fused in-frame to each other. The non-MBSPX polypeptide can be fused to the N-terminus or C-terminus of the MBSPX polypeptide.

In yet another embodiment, the fusion protein is a GST-MBSPX fusion protein in which the MBSPX sequences are fused to the C-terminus of the GST (*i.e.*, glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant MBSPX.

In another embodiment, the fusion protein is an MBSPX protein containing a heterologous signal sequence at its N-terminus. For example, the native MBSPX signal sequence can be removed and replaced with a signal sequence from another protein. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of MBSPX can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is an MBSPX-immunoglobulin fusion protein in which the MBSPX are fused to sequences derived from a member of the immunoglobulin protein family. The MBSPX-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between an MBSPX ligand and an MBSPX protein on the surface of a cell, to thereby suppress MBSPX-mediated signal transduction *in vivo*. The MBSPX-immunoglobulin fusion proteins can be used to affect the bioavailability of an MBSPX cognate ligand. Inhibition of the MBSPX ligand/MBSPX interaction are useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as

modulating (*e.g.* promoting or inhibiting) cell survival. Moreover, the MBSPX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-MBSPX antibodies in a subject, to purify MBSPX ligands, and in screening assays to identify molecules that inhibit the interaction of MBSPX with an MBSPX ligand.

An MBSPX chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Ausubel *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). An MBSPX-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the MBSPX protein.

MBSPX agonists and antagonists

The present invention also pertains to variants of the MBSPX proteins that function as either MBSPX agonists (mimetics) or as MBSPX antagonists. Variants of the MBSPX protein can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of the MBSPX protein. An agonist of the MBSPX protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the MBSPX protein. An antagonist of the MBSPX protein can inhibit one or more of the activities of the naturally occurring form of the MBSPX protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the MBSPX protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of

the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the MBSPX proteins.

Variants of the MBSPX protein that function as either MBSPX agonists (mimetics) or as MBSPX antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of the MBSPX protein for MBSPX protein agonist or antagonist activity. In one embodiment, a variegated library of MBSPX variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of MBSPX variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential MBSPX sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of MBSPX sequences therein. There are a variety of methods which can be used to produce libraries of potential MBSPX variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential MBSPX sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu Rev Biochem* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucl Acid Res* 11:477.

Polypeptide libraries

In addition, libraries of fragments of the MBSPX protein coding sequence can be used to generate a variegated population of MBSPX fragments for screening and subsequent selection of variants of an MBSPX protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an MBSPX coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can

be derived which encodes N-terminal and internal fragments of various sizes of the MBSPX protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of MBSPX proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recrusive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify MBSPX variants (Arkin and Yourvan (1992) PNAS 89:7811-7815; Delgrave *et al.* (1993) Protein Engineering 6:327-331).

Anti-MBSPX antibodies

An isolated MBSPX protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind MBSPX using standard techniques for polyclonal and monoclonal antibody preparation. The full-length MBSPX protein can be used or, alternatively, the invention provides antigenic peptide fragments of MBSPX for use as immunogens. The antigenic peptide of MBSPX comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24 and encompasses an epitope of MBSPX such that an antibody raised against the peptide forms a specific immune complex with MBSPX. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues. Preferred epitopes encompassed by the antigenic peptide are regions of MBSPX that are located on the surface of the protein, *e.g.*, hydrophilic regions.

As disclosed herein, MBSPX protein sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen, such as MBSPX. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} and $F_{(ab')2}$ fragments, and an F_{ab} expression library. In a specific embodiment, antibodies to human MBSPX proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to an MBSPX protein sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24, or derivative, fragment, analog or homolog thereof.

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, or a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, recombinantly expressed MBSPX protein or a chemically synthesized MBSPX polypeptide. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium parvum*, or similar immunostimulatory agents. If desired, the antibody molecules directed against MBSPX can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction.

The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of MBSPX. A monoclonal antibody composition thus typically displays a single binding affinity for a particular MBSPX protein with which it immunoreacts. For preparation of monoclonal antibodies directed towards a particular MBSPX protein, or derivatives, fragments, analogs or homologs thereof,

any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (see Kohler & Milstein, 1975 *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (see Kozbor, *et al.*, 1983 *Immunol Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, *et al.*, 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, *et al.*, 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (see Cole, *et al.*, 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96).

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to an MBSPX protein (see *e.g.*, U.S. Patent No. 4,946,778). In addition, methodologies can be adapted for the construction of F_{ab} expression libraries (see *e.g.*, Huse, *et al.*, 1989 *Science* 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for an MBSPX protein or derivatives, fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. See *e.g.*, U.S. Patent No. 5,225,539. Antibody fragments that contain the idiotypes to an MBSPX protein may be produced by techniques known in the art including, but not limited to: (*i*) an $F_{(ab')2}$ fragment produced by pepsin digestion of an antibody molecule; (*ii*) an F_{ab} fragment generated by reducing the disulfide bridges of an $F_{(ab')2}$ fragment; (*iii*) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (*iv*) F_v fragments.

Additionally, recombinant anti-MBSPX antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT International Application No. PCT/US86/02269; European Patent Application No. 184,187; European Patent Application No. 171,496; European Patent Application No. 173,494; PCT International Publication No. WO 86/01533; U.S. Pat. No. 4,816,567; European Patent Application No.

125,023; Better *et al.*(1988) *Science* 240:1041-1043; Liu *et al.* (1987) *PNAS* 84:3439-3443; Liu *et al.* (1987) *J Immunol.* 139:3521-3526; Sun *et al.* (1987) *PNAS* 84:214-218; Nishimura *et al.* (1987) *Cancer Res* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; Shaw *et al.* (1988) *J Natl Cancer Inst* 80:1553-1559); Morrison(1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; U.S. Pat. No. 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J Immunol* 141:4053-4060.

In one embodiment, methodologies for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art.

Anti-MBSPX antibodies may be used in methods known within the art relating to the localization and/or quantitation of an MBSPX protein (*e.g.,* for use in measuring levels of the MBSPX protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies for MBSPX proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antibody derived binding domain, are utilized as pharmacologically-active compounds [hereinafter "Therapeutics"].

An anti-MBSPX antibody (*e.g.*, monoclonal antibody) can be used to isolate MBSPX by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-MBSPX antibody can facilitate the purification of natural MBSPX from cells and of recombinantly produced MBSPX expressed in host cells. Moreover, an anti-MBSPX antibody can be used to detect MBSPX protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the MBSPX protein. Anti-MBSPX antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group

complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

MBSPX Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding MBSPX protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is

intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, MBSPX proteins, mutant forms of MBSPX, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of MBSPX in prokaryotic or eukaryotic cells. For example, MBSPX can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (1) to increase expression of recombinant protein; (2) to increase the solubility of the recombinant protein; and (3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to

enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. See, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the MBSPX expression vector is a yeast expression vector. Examples of vectors for expression in yeast S. cerivisae include pYepSec1 (Baldari, *et al.*, (1987) *EMBO J* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

Alternatively, MBSPX can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith *et al.* (1983) *Mol Cell Biol* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J*

6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells. See, *e.g.*, Chapters 16 and 17 of Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv Immunol* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to MBSPX mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression

of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub *et al.*, "Antisense RNA as a molecular tool for genetic analysis," Reviews--Trends in Genetics, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, MBSPX protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host

cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding MBSPX or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) MBSPX protein. Accordingly, the invention further provides methods for producing MBSPX protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding MBSPX has been introduced) in a suitable medium such that MBSPX protein is produced. In another embodiment, the method further comprises isolating MBSPX from the medium or the host cell.

Transgenic animals

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which MBSPX-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous MBSPX sequences have been introduced into their genome or homologous recombinant animals in which endogenous MBSPX sequences have been altered. Such animals are useful for studying the function and/or activity of MBSPX and for identifying and/or evaluating modulators of MBSPX activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous MBSPX gene has

been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing MBSPX-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The human MBSPX cDNA sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of the human MBSPX gene, such as a mouse MBSPX gene, can be isolated based on hybridization to the human MBSPX cDNA (described further above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the MBSPX transgene to direct expression of MBSPX protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Pat. Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan 1986, In: MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the MBSPX transgene in its genome and/or expression of MBSPX mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding MBSPX can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a MBSPX gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the MBSPX gene. The MBSPX gene can be a human gene (*e.g.*, the cDNA of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23) but more preferably, is a non-human homologue of a human MBSPX gene. For example, a mouse homologue of human MBSPX gene of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 can be used to construct a homologous recombination vector suitable for altering an

endogenous MBSPX gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous MBSPX gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous MBSPX gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous MBSPX protein). In the homologous recombination vector, the altered portion of the MBSPX gene is flanked at its 5' and 3' ends by additional nucleic acid of the MBSPX gene to allow for homologous recombination to occur between the exogenous MBSPX gene carried by the vector and an endogenous MBSPX gene in an embryonic stem cell. The additional flanking MBSPX nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector. See *e.g.*, Thomas *et al.* (1987) *Cell* 51:503 for a description of homologous recombination vectors. The vector is introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced MBSPX gene has homologously recombined with the endogenous MBSPX gene are selected (see *e.g.*, Li *et al.* (1992) *Cell* 69:915).

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. See *e.g.*, Bradley 1987, In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley (1991) *Curr Opin Biotechnol* 2:823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-human animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the

cre/loxP recombinase system, see, *e.g.*, Lakso *et al.* (1992) *PNAS* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut *et al.* (1997) *Nature* 385:810-813. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G_0 phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

Pharmaceutical Compositions

The MBSPX nucleic acid molecules, MBSPX proteins, and anti-MBSPX antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the

composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, an MBSPX protein or anti-MBSPX antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and

include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Pat. No. 5,328,470) or by stereotactic

injection (see *e.g.*, Chen *et al.* (1994) *PNAS* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Uses and Methods of the Invention

The isolated nucleic acid molecules of the invention can be used to express MBSPX protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect MBSPX mRNA (*e.g.*, in a biological sample) or a genetic lesion in an MBSPX gene, and to modulate MBSPX activity, as described further below. In addition, the MBSPX proteins can be used to screen drugs or compounds that modulate the MBSPX activity or expression as well as to treat disorders characterized by insufficient or excessive production of MBSPX protein or production of MBSPX protein forms that have decreased or aberrant activity compared to MBSPX wild type protein (*e.g.* proliferative disorders such as cancer or preclampsia, immune system disorders and inflammation, neurological disorders, and skin and muscle abnormalities). In addition, the anti-MBSPX antibodies of the invention can be used to detect and isolate MBSPX proteins and modulate MBSPX activity.

This invention further pertains to novel agents identified by the above described screening assays and uses thereof for treatments as described herein.

Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to MBSPX proteins or have a stimulatory or inhibitory effect on, for example, MBSPX expression or MBSPX activity.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a membrane-bound form of an MBSPX

protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc Natl Acad Sci U.S.A.* 90:6909; Erb *et al.* (1994) *Proc Natl Acad Sci U.S.A.* 91:11422; Zuckermann *et al.* (1994) *J Med Chem* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew Chem Int Ed Engl* 33:2059; Carell *et al.* (1994) *Angew Chem Int Ed Engl* 33:2061; and Gallop *et al.* (1994) *J Med Chem* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), on chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner U.S. Pat. No. 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 249:404-406; Cwirla *et al.* (1990) *Proc Natl Acad Sci U.S.A.* 87:6378-6382; Felici (1991) *J Mol Biol* 222:301-310; Ladner above.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of MBSPX protein, or a biologically active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to an MBSPX protein is determined. The cell, for example, can be of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the MBSPX protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the MBSPX protein or biologically active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting.

Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of MBSPX protein, or a biologically active portion thereof, on the cell surface with a known compound which binds MBSPX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with MBSPX protein, wherein determining the ability of the test compound to interact with an MBSPX protein comprises determining the ability of the test compound to preferentially bind to MBSPX or a biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of MBSPX protein, or a biologically active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the MBSPX protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of MBSPX or a biologically active portion thereof can be accomplished, for example, by determining the ability of the MBSPX protein to bind to or interact with an MBSPX target molecule. As used herein, a "target molecule" is a molecule with which an MBSPX protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses an MBSPX protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. An MBSPX target molecule can be a non-MBSPX molecule or an MBSPX protein or polypeptide of the present invention. In one embodiment, an MBSPX target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.* a signal generated by binding of a compound to a membrane-bound MBSPX molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with MBSPX.

Determining the ability of the MBSPX protein to bind to or interact with an MBSPX target molecule can be accomplished by one of the methods described above for determining

direct binding. In one embodiment, determining the ability of the MBSPX protein to bind to or interact with an MBSPX target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca^{2+}, diacylglycerol, IP_3, etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising an MBSPX-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the present invention is a cell-free assay comprising contacting an MBSPX protein or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to the MBSPX protein or biologically active portion thereof. Binding of the test compound to the MBSPX protein can be determined either directly or indirectly as described above. In one embodiment, the assay comprises contacting the MBSPX protein or biologically active portion thereof with a known compound which binds MBSPX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an MBSPX protein, wherein determining the ability of the test compound to interact with an MBSPX protein comprises determining the ability of the test compound to preferentially bind to MBSPX or biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-free assay comprising contacting MBSPX protein or biologically active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the MBSPX protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of MBSPX can be accomplished, for example, by determining the ability of the MBSPX protein to bind to an MBSPX target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of MBSPX can be accomplished by determining the ability of the MBSPX protein further modulate an MBSPX target molecule. For example,

the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described.

In yet another embodiment, the cell-free assay comprises contacting the MBSPX protein or biologically active portion thereof with a known compound which binds MBSPX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an MBSPX protein, wherein determining the ability of the test compound to interact with an MBSPX protein comprises determining the ability of the MBSPX protein to preferentially bind to or modulate the activity of an MBSPX target molecule.

The cell-free assays of the present invention are amenable to use of both the soluble form or the membrane-bound form of MBSPX. In the case of cell-free assays comprising the membrane-bound form of MBSPX, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of MBSPX is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$, 3-(3-cholamidopropyl)dimethylamminiol-1-propane sulfonate (CHAPS), 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either MBSPX or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to MBSPX, or interaction of MBSPX with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-MBSPX fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test

compound and either the non-adsorbed target protein or MBSPX protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of MBSPX binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either MBSPX or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated MBSPX or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with MBSPX or target molecules, but which do not interfere with binding of the MBSPX protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or MBSPX trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the MBSPX or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the MBSPX or target molecule.

In another embodiment, modulators of MBSPX expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of MBSPX mRNA or protein in the cell is determined. The level of expression of MBSPX mRNA or protein in the presence of the candidate compound is compared to the level of expression of MBSPX mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of MBSPX expression based on this comparison. For example, when expression of MBSPX mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of MBSPX mRNA or protein expression. Alternatively, when expression of MBSPX mRNA or protein is less (statistically significantly

less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of MBSPX mRNA or protein expression. The level of MBSPX mRNA or protein expression in the cells can be determined by methods described herein for detecting MBSPX mRNA or protein.

In yet another aspect of the invention, the MBSPX proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (see, *e.g.*, U.S. Pat. No. 5,283,317; Zervos *et al.* (1993) Cell 72:223-232; Madura *et al.* (1993) J Biol Chem 268:12046-12054; Bartel *et al.* (1993) Biotechniques 14:920-924; Iwabuchi *et al.* (1993) Oncogene 8:1693-1696; and Brent WO94/10300), to identify other proteins that bind to or interact with MBSPX ("MBSPX-binding proteins" or "MBSPX-bps") and modulate MBSPX activity. Such MBSPX-binding proteins are also likely to be involved in the propagation of signals by the MBSPX proteins as, for example, upstream or downstream elements of the MBSPX pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for MBSPX is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a MBSPX-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close MBSPXimity. This MBSPXimity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with MBSPX.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the MBSPX, sequences, described herein, can be used to map the location of the MBSPX genes, respectively, on a chromosome. The mapping of the MBSPX sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, MBSPX genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the MBSPX sequences. Computer analysis of the MBSPX, sequences can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the MBSPX sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but in which human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio *et al.* (1983) *Science*

220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the MBSPX sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical like colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases, will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, HUMAN CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in McKusick, MENDELIAN INHERITANCE IN MAN, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through

linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland *et al.* (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the MBSPX gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

Tissue Typing

The MBSPX sequences of the present invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Pat. No. 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the MBSPX sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The MBSPX sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs

with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. For example, the noncoding sequences of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as the nucleic acid seqences that code for the amino acid sequences of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trails are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining MBSPX protein and/or nucleic acid expression as well as MBSPX activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant MBSPX expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with MBSPX protein, nucleic acid expression or activity. For example, mutations in an MBSPX gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with MBSPX protein, nucleic acid expression or activity.

Another aspect of the invention provides methods for determining MBSPX protein, nucleic acid expression or MBSPX activity in an individual to thereby select appropriate

therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent).

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of MBSPX in clinical trials.

These and other agents are described in further detail in the following sections.

Diagnostic Assays

An exemplary method for detecting the presence or absence of MBSPX in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting MBSPX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes MBSPX protein such that the presence of MBSPX is detected in the biological sample. An agent for detecting MBSPX mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to MBSPX mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length MBSPX nucleic acid, such as the nucleic acid of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23 , or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to MBSPX mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting MBSPX protein is an antibody capable of binding to MBSPX protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled

streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect MBSPX mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of MBSPX mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of MBSPX protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of MBSPX genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of MBSPX protein include introducing into a subject a labeled anti-MBSPX antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting MBSPX protein, mRNA, or genomic DNA, such that the presence of MBSPX protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of MBSPX protein, mRNA or genomic DNA in the control sample with the presence of MBSPX protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of MBSPX in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting MBSPX protein or mRNA in a biological sample; means for determining the amount of MBSPX in the sample; and means for comparing the amount of MBSPX in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect MBSPX protein or nucleic acid.

Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant MBSPX expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with MBSPX protein, nucleic acid expression or activity such as cancer or fibrotic disorders. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant MBSPX expression or activity in which a test sample is obtained from a subject and MBSPX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of MBSPX protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant MBSPX expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant MBSPX expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder, such as cancer or preclampsia. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant MBSPX expression or activity in which a test sample is obtained and MBSPX protein or nucleic acid is detected (*e.g.*, wherein the presence of MBSPX protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant MBSPX expression or activity.)

The methods of the invention can also be used to detect genetic lesions in an MBSPX gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a

genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding an MBSPX-protein, or the mis-expression of the MBSPX gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of (1) a deletion of one or more nucleotides from an MBSPX gene; (2) an addition of one or more nucleotides to an MBSPX gene; (3) a substitution of one or more nucleotides of an MBSPX gene, (4) a chromosomal rearrangement of an MBSPX gene; (5) an alteration in the level of a messenger RNA transcript of an MBSPX gene, (6) aberrant modification of an MBSPX gene, such as of the methylation pattern of the genomic DNA, (7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of an MBSPX gene, (8) a non-wild type level of an MBSPX-protein, (9) allelic loss of an MBSPX gene, and (10) inappropriate post-translational modification of an MBSPX-protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in an MBSPX gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Pat. Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *PNAS* 91:360-364), the latter of which can be particularly useful for detecting point mutations in an MBSPX-gene (see Abravaya *et al.* (1995) *Nucl Acids Res* 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to an MBSPX gene under conditions such that hybridization and amplification of the MBSPX gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli *et al.*, 1990, *Proc Natl Acad Sci USA* 87:1874-1878), transcriptional amplification system (Kwoh, *et al.*, 1989, *Proc Natl Acad Sci USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al*, 1988, *BioTechnology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in an MBSPX gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicate mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Pat. No. 5,493,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in MBSPX can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin *et al.* (1996) *Human Mutation* 7: 244-255; Kozal *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in MBSPX can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin *et al.* above. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the MBSPX gene and detect mutations by comparing the

sequence of the sample MBSPX with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert (1977) *PNAS* 74:560 or Sanger (1977) *PNAS* 74:5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (Naeve *et al.*, (1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publ. No. WO 94/16101; Cohen *et al.* (1996) *Adv Chromatogr* 36:127-162; and Griffin *et al.* (1993) *Appl Biochem Biotechnol* 38:147-159).

Other methods for detecting mutations in the MBSPX gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type MBSPX sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al* (1988) *Proc Natl Acad Sci USA* 85:4397; Saleeba *et al* (1992) *Methods Enzymol* 217:286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in MBSPX cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on an MBSPX sequence, *e.g.*, a wild-type MBSPX

sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Pat. No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in MBSPX genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl Acad Sci USA*: 86:2766, see also Cotton (1993) *Mutat Res* 285:125-144; Hayashi (1992) *Genet Anal Tech Appl* 9:73-79). Single-stranded DNA fragments of sample and control MBSPX nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of apMBSPXimately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are

hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al* (1992) *Mol Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc Natl Acad Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving an MBSPX gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which MBSPX is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on MBSPX activity (*e.g.*, MBSPX gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (*e.g.*, cancer or immune disorders, neurological disorders, muscular dystrophy, or epidermolysis bullosa

simplex) associated with aberrant MBSPX activity. In conjunction with such treatment, the pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.*, drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of MBSPX protein, expression of MBSPX nucleic acid, or mutation content of MBSPX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, *Clin Exp Pharmacol Physiol*, 1996, 23:983-985 and Linder, *Clin Chem*, 1997, 43:254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor

metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme is the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of MBSPX protein, expression of MBSPX nucleic acid, or mutation content of MBSPX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an MBSPX modulator, such as a modulator identified by one of the exemplary screening assays described herein.

Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of MBSPX (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase MBSPX gene expression, protein levels, or upregulate MBSPX activity, can be monitored in clinical trails of subjects exhibiting decreased MBSPX gene expression, protein levels, or downregulated MBSPX activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease MBSPX gene expression, protein levels, or downregulate MBSPX activity, can be monitored in clinical trails of subjects exhibiting increased MBSPX gene expression, protein levels, or upregulated MBSPX activity. In such clinical trials, the expression or activity of MBSPX and, preferably, other genes that have been

implicated in, for example, a cellular proliferation disorder can be used as a "read out" or markers of the immune responsiveness of a particular cell.

For example, and not by way of limitation, genes, including MBSPX, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates MBSPX activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of MBSPX and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of MBSPX or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, peptidomimetic, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (*i*) obtaining a pre-administration sample from a subject prior to administration of the agent; (*ii*) detecting the level of expression of an MBSPX protein, mRNA, or genomic DNA in the preadministration sample; (*iii*) obtaining one or more post-administration samples from the subject; (*iv*) detecting the level of expression or activity of the MBSPX protein, mRNA, or genomic DNA in the post-administration samples; (*v*) comparing the level of expression or activity of the MBSPX protein, mRNA, or genomic DNA in the pre-administration sample with the MBSPX protein, mRNA, or genomic DNA in the post administration sample or samples; and (*vi*) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of MBSPX to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of MBSPX to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant MBSPX expression or activity.

Disorders

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (i.e., reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, (i) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (ii) antibodies to an aforementioned peptide; (iii) nucleic acids encoding an aforementioned peptide; (iv) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (i.e., due to a heterologous insertion within the coding sequences of coding sequences to an aforementioned peptide) that are utilized to "knockout" endogenous function of an aforementioned peptide by homologous recombination (see, e.g., Capecchi, 1989. Science 244: 1288-1292); or (v) modulators (i.e., inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (i.e., are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (e.g., from biopsy tissue) and assaying it in vitro for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of an aforementioned peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (e.g., by Western blot analysis, immunoprecipitation followed by

sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (e.g., Northern assays, dot blots, in situ hybridization, etc.).

Prophylactic Methods

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant MBSPX expression or activity, by administering to the subject an agent that modulates MBSPX expression or at least one MBSPX activity. Subjects at risk for a disease that is caused or contributed to by aberrant MBSPX expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the MBSPX aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of MBSPX aberrancy, for example, an MBSPX agonist or MBSPX antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein. The prophylactic methods of the present invention are further discussed in the following subsections.

Therapeutic Methods

Another aspect of the invention pertains to methods of modulating MBSPX expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of MBSPX protein activity associated with the cell. An agent that modulates MBSPX protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of an MBSPX protein, a peptide, an MBSPX peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more MBSPX protein activity. Examples of such stimulatory agents include active MBSPX protein and a nucleic acid molecule encoding MBSPX that has been introduced into the cell. In another embodiment, the agent inhibits one or more MBSPX protein activity. Examples of such inhibitory agents include antisense MBSPX nucleic acid molecules and anti-MBSPX antibodies. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of

treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of an MBSPX protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) MBSPX expression or activity. In another embodiment, the method involves administering an MBSPX protein or nucleic acid molecule as therapy to compensate for reduced or aberrant MBSPX expression or activity.

Stimulation of MBSPX activity is desirable in situations in which MBSPX is abnormally downregulated and/or in which increased MBSPX activity is likely to have a beneficial effect. One example of such a situation is where a subject has a disorder characterized by aberrant cell proliferation and/or differentiation (*e.g.*, cancer). Another example of such a situation is where the subject has a gestational disease (*e.g.*, preclampsia).

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

This invention is further illustrated by the following examples, which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference.

DISEASE PATHWAYS

Determination of the Biological Effect of the Therapeutic

In various embodiments of the present invention, suitable *in vitro* or *in vivo* assays are utilized to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts

the desired effect upon the cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model systems known in the art may be used prior to administration to human subjects.

Malignancies

An aforementioned protein is involved in the regulation of cell proliferation. Accordingly, Therapeutics of the present invention are useful in the therapeutic or prophylactic treatment of diseases or disorders that are associated with cell hyperproliferation and/or loss of control of cell proliferation (*e.g.*, cancers, malignancies and tumors). For a review of such hyperproliferation disorders, see *e.g.*, Fishman, *et al.*, 1985. MEDICINE, 2nd ed., J.B. Lippincott Co., Philadelphia, PA.

Therapeutics of the present invention may be assayed by any method known within the art for efficacy in treating or preventing malignancies and related disorders. Such assays include, but are not limited to, *in vitro* assays utilizing transformed cells or cells derived from the patient's tumor, as well as *in vivo* assays using animal models of cancer or malignancies. Potentially effective Therapeutics are those that, for example, inhibit the proliferation of tumor-derived or transformed cells in culture or cause a regression of tumors in animal models, in comparison to the controls.

In the practice of the present invention, once a malignancy or cancer has been shown to be amenable to treatment by modulating (*i.e.*, inhibiting, antagonizing or agonizing) activity, that cancer or malignancy may subsequently be treated or prevented by the administration of a Therapeutic that serves to modulate protein function.

Premalignant conditions

The Therapeutics of the present invention that are effective in the therapeutic or prophylactic treatment of cancer or malignancies may also be administered for the treatment of pre-malignant conditions and/or to prevent the progression of a pre-malignancy to a neoplastic or malignant state. Such prophylactic or therapeutic use is indicated in conditions known or

suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia or, most particularly, dysplasia has occurred. For a review of such abnormal cell growth see *e.g.*, Robbins & Angell, 1976. BASIC PATHOLOGY, 2nd ed., W.B. Saunders Co., Philadelphia, PA.

Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in its structure or function. For example, it has been demonstrated that endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of mature or fully differentiated cell substitutes for another type of mature cell. Metaplasia may occur in epithelial or connective tissue cells. Dysplasia is generally considered a precursor of cancer, and is found mainly in the epithelia. Dysplasia is the most disorderly form of non-neoplastic cell growth, and involves a loss in individual cell uniformity and in the architectural orientation of cells. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively, or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed or malignant phenotype displayed either *in vivo* or *in vitro* within a cell sample derived from a patient, is indicative of the desirability of prophylactic/therapeutic administration of a Therapeutic that possesses the ability to modulate activity of An aforementioned protein. Characteristics of a transformed phenotype include, but are not limited to: (*i*) morphological changes; (*ii*) looser substratum attachment; (*iii*) loss of cell-to-cell contact inhibition; (*iv*) loss of anchorage dependence; (*v*) protease release; (*vi*) increased sugar transport; (*vii*) decreased serum requirement; (*viii*) expression of fetal antigens, (*ix*) disappearance of the 250 kDal cell-surface protein, and the like. See *e.g.*, Richards, *et al.*, 1986. MOLECULAR PATHOLOGY, W.B. Saunders Co., Philadelphia, PA.

In a specific embodiment of the present invention, a patient that exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of a Therapeutic: (*i*) a chromosomal translocation associated with a

malignancy (*e.g.*, the Philadelphia chromosome (*bcr/abl*) for chronic myelogenous leukemia and t(14;18) for follicular lymphoma, etc.); (*ii*) familial polyposis or Gardner's syndrome (possible forerunners of colon cancer); (*iii*) monoclonal gammopathy of undetermined significance (a possible precursor of multiple myeloma) and (*iv*) a first degree kinship with persons having a cancer or pre-cancerous disease showing a Mendelian (genetic) inheritance pattern (*e.g.*, familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, medullary thyroid carcinoma with amyloid production and pheochromocytoma, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia and Bloom's syndrome).

In another embodiment, a Therapeutic of the present invention is administered to a human patient to prevent the progression to breast, colon, lung, pancreatic, or uterine cancer, or melanoma or sarcoma.

Hyperproliferative and dysproliferative disorders

In one embodiment of the present invention, a Therapeutic is administered in the therapeutic or prophylactic treatment of hyperproliferative or benign dysproliferative disorders. The efficacy in treating or preventing hyperproliferative diseases or disorders of a Therapeutic of the present invention may be assayed by any method known within the art. Such assays include *in vitro* cell proliferation assays, *in vitro* or *in vivo* assays using animal models of hyperproliferative diseases or disorders, or the like. Potentially effective Therapeutics may, for example, promote cell proliferation in culture or cause growth or cell proliferation in animal models in comparison to controls.

Specific embodiments of the present invention are directed to the treatment or prevention of cirrhosis of the liver (a condition in which scarring has overtaken normal liver regeneration processes); treatment of keloid (hypertrophic scar) formation causing disfiguring of the skin in which the scarring process interferes with normal renewal; psoriasis (a common skin condition characterized by excessive proliferation of the skin and delay in proper cell fate

determination); benign tumors; fibrocystic conditions and tissue hypertrophy (*e.g.*, benign prostatic hypertrophy).

Neurodegenerative disorders

MBSPX has been implicated in the deregulation of cellular maturation and apoptosis, which are both characteristic of neurodegenerative disease. Accordingly, Therapeutics of the invention, particularly but not limited to those that modulate (or supply) activity of an aforementioned protein, may be effective in treating or preventing neurodegenerative disease. Therapeutics of the present invention that modulate the activity of an aforementioned protein involved in neurodegenerative disorders can be assayed by any method known in the art for efficacy in treating or preventing such neurodegenerative diseases and disorders. Such assays include *in vitro* assays for regulated cell maturation or inhibition of apoptosis or *in vivo* assays using animal models of neurodegenerative diseases or disorders, or any of the assays described below. Potentially effective Therapeutics, for example but not by way of limitation, promote regulated cell maturation and prevent cell apoptosis in culture, or reduce neurodegeneration in animal models in comparison to controls.

Once a neurodegenerative disease or disorder has been shown to be amenable to treatment by modulation activity, that neurodegenerative disease or disorder can be treated or prevented by administration of a Therapeutic that modulates activity. Such diseases include all degenerative disorders involved with aging, especially osteoarthritis and neurodegenerative disorders.

Disorders related to organ transplantation

MBSPX has been implicated in disorders related to organ transplantation, in particular but not limited to organ rejection. Therapeutics of the invention, particularly those that modulate (or supply) activity, may be effective in treating or preventing diseases or disorders related to organ transplantation. Therapeutics of the invention (particularly Therapeutics that modulate the levels or activity of an aforementioned protein) can be assayed by any method known in the art for efficacy in treating or preventing such diseases and disorders related to organ transplantation. Such assays include *in vitro* assays for using cell culture models as described below, or *in vivo* assays using animal models of diseases and disorders related to

organ transplantation, see *e.g.*, below. Potentially effective Therapeutics, for example but not by way of limitation, reduce immune rejection responses in animal models in comparison to controls.

Accordingly, once diseases and disorders related to organ transplantation are shown to be amenable to treatment by modulation of activity, such diseases or disorders can be treated or prevented by administration of a Therapeutic that modulates activity.

Cardiovascular Disease

MBSPX has been implicated in cardiovascular disorders, including in atherosclerotic plaque formation. Diseases such as cardiovascular disease, including cerebral thrombosis or hemorrhage, ischemic heart or renal disease, peripheral vascular disease, or thrombosis of other major vessel, and other diseases, including diabetes mellitus, hypertension, hypothyroidism, cholesterol ester storage disease, systemic lupus erythematosus, homocysteinemia, and familial protein or lipid processing diseases, and the like, are either directly or indirectly associated with atherosclerosis. Accordingly, Therapeutics of the invention, particularly those that modulate (or supply) activity or formation may be effective in treating or preventing atherosclerosis-associated diseases or disorders. Therapeutics of the invention (particularly Therapeutics that modulate the levels or activity) can be assayed by any method known in the art, including those described below, for efficacy in treating or preventing such diseases and disorders.

A vast array of animal and cell culture models exist for processes involved in atherosclerosis. A limited and non-exclusive list of animal models includes knockout mice for premature atherosclerosis (Kurabayashi and Yazaki, 1996, Int. Angiol. 15: 187-194), transgenic mouse models of atherosclerosis (Kappel *et al.*, 1994, FASEB J. 8: 583-592), antisense oligonucleotide treatment of animal models (Callow, 1995, Curr. Opin. Cardiol. 10: 569-576), transgenic rabbit models for atherosclerosis (Taylor, 1997, Ann. N.Y. Acad. Sci 811: 146-152), hypercholesterolemic animal models (Rosenfeld, 1996, Diabetes Res. Clin. Pract. 30 Suppl.: 1-11), hyperlipidemic mice (Paigen *et al.*, 1994, Curr. Opin. Lipidol. 5: 258-264), and inhibition of lipoxygenase in animals (Sigal *et al.*, 1994, Ann. N.Y. Acad. Sci. 714: 211-224). In addition, *in vitro* cell models include but are not limited to monocytes

exposed to low density lipoprotein (Frostegard *et al.*, 1996, Atherosclerosis 121: 93-103), cloned vascular smooth muscle cells (Suttles *et al.*, 1995, Exp. Cell Res. 218: 331-338), endothelial cell-derived chemoattractant exposed T cells (Katz *et al.*, 1994, J. Leukoc. Biol. 55: 567-573), cultured human aortic endothelial cells (Farber *et al.*, 1992, Am. J. Physiol. 262: H1088-1085), and foam cell cultures (Libby *et al.*, 1996, Curr Opin Lipidol 7: 330-335). Potentially effective Therapeutics, for example but not by way of limitation, reduce foam cell formation in cell culture models, or reduce atherosclerotic plaque formation in hypercholesterolemic mouse models of atherosclerosis in comparison to controls.

Accordingly, once an atherosclerosis-associated disease or disorder has been shown to be amenable to treatment by modulation of activity or formation, that disease or disorder can be treated or prevented by administration of a Therapeutic that modulates activity.

Cytokine and Cell Proliferation/Differentiation Activity

An MBSPX protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods: Assays for T-cell or thymocyte proliferation include without limitation those described in: CURRENT PROTOCOLS IN IMMUNOLOGY, Ed by Coligan *et al.*, Greene Publishing Associates and Wiley-Interscience (Chapter 3 and Chapter 7); Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Bertagnoili *et al.*, *J Immunol* 145:1706-1712, 1990; Bertagnolli *et al.*, *Cell Immunol* 133:327-341, 1991; Bertagnolli, *et al.*, *J Immunol* 149:3778-3783, 1992; Bowman *et al.*, *J Immunol* 152:1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described by Kruisbeek and Shevach, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds., Vol 1, pp. 3.12.1-14, John Wiley and Sons, Toronto 1994; and by Schreiber, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan eds., Vol 1, pp. 6.8.1-8, John Wiley and Sons, Toronto 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described by Bottomly *et al.*, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds., Vol 1, pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto 1991; deVries *et al.*, *J Exp Med* 173:1205-1211, 1991; Moreau *et al.*, *Nature* 336:690-692, 1988; Greenberger *et al.*, *Proc Natl Acad Sci U.S.A.* 80:2931-2938, 1983; Nordan, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds., Vol 1, pp. 6.6.1-5, John Wiley and Sons, Toronto 1991; Smith *et al.*, *Proc Natl Acad Sci U.S.A.* 83:1857-1861, 1986; Measurement of human Interleukin 11-Bennett, *et* al. In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds., Vol 1, pp. 6.15.1 John Wiley and Sons, Toronto 1991; Ciarletta, *et al.*, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds., Vol 1, pp. 6.13.1, John Wiley and Sons, Toronto 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds., Greene Publishing Associates and Wiley-Interscience (Chapter 3, Chapter 6, Chapter 7); Weinberger *et al.*, *Proc Natl Acad Sci USA* 77:6091-6095, 1980; Weinberger *et al.*, *Eur J Immun* 11:405-411, 1981; Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Takai *et al.*, *J Immunol* 140:508-512, 1988.

Immune Stimulating or Suppressing Activity

An MBSPX protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein are useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), *e.g.*, in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or

be caused by viral (*e.g.*, HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania species, malaria species, and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, *i.e.*, in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention.

Using the proteins of the invention it may also be possible to immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or energy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon re-exposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as, for example, B7), *e.g.*, preventing high level lymphokine synthesis by activated T cells, are useful in situations of tissue, skin and

organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (*e.g.*, B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to energize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins *in vivo* as described in Lenschow *et al.*, Science 257:789-792 (1992) and Turka *et al.*, Proc Natl Acad Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., FUNDAMENTAL IMMUNOLOGY, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function *in vivo* on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and auto-antibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block

costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of auto-antibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythematosis in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., FUNDAMENTAL IMMUNOLOGY, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function are useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells *in vitro* with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the *in vitro* activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells *in vivo*.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) are useful in the induction of tumor immunity. Tumor cells

(*e.g.*, sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected *ex vivo* with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection *in vivo*.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (*e.g.*, a cytoplasmic-domain truncated portion) of an MHC class I a chain protein and b $_2$ microglobulin protein or an MHC class II a chain protein and an MHC class II b chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (*e.g.*, B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods: Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Greene Publishing Associates and Wiley-Interscience (Chapter 3, Chapter 7); Herrmann *et al.*,

Proc Natl Acad Sci USA 78:2488-2492, 1981; Herrmann *et al.*, *J Immunol* 128:1968-1974, 1982; Handa *et al.*, *J Immunol* 135:1564-1572, 1985; Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Takai *et al.*, *J Immunol* 140:508-512, 1988; Herrmann *et al.*, *Proc Natl Acad Sci USA* 78:2488-2492, 1981; Herrmann *et al.*, *J Immunol* 128:1968-1974, 1982; Handa *et al.*, *J Immunol* 135:1564-1572, 1985; Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Bowman *et al.*, *J Virology* 61:1992-1998; Takai *et al.*, *J Immunol* 140:508-512, 1988; Bertagnolli *et al.*, *Cell Immunol* 133:327-341, 1991; Brown *et al.*, *J Immunol* 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, *J Immunol* 144:3028-3033, 1990; and Mond and Brunswick In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds., Vol 1, pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Greene Publishing Associates and Wiley-Interscience (Chapter 3, Chapter 7); Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Takai *et al.*, *J Immunol* 140:508-512, 1988; Bertagnolli *et al.*, *J Immunol* 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery *et al.*, *J Immunol* 134:536-544, 1995; Inaba *et al.*, *J Exp Med* 173:549-559, 1991; Macatonia *et al.*, *J Immunol* 154:5071-5079, 1995; Porgador *et al.*, *J Exp Med* 182:255-260, 1995; Nair *et al.*, *J Virol* 67:4062-4069, 1993; Huang *et al.*, *Science* 264:961-965, 1994; Macatonia *et al.*, *J Exp Med* 169:1255-1264, 1989; Bhardwaj *et al.*, *J Clin Investig* 94:797-807, 1994; and Inaba *et al.*, *J Exp Med* 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz *et al.*, *Cytometry* 13:795-808, 1992; Gorczyca *et al.*, *Leukemia* 7:659-670, 1993; Gorczyca *et al.*, *Cancer Res*

53:1945-1951, 1993; Itoh *et al.*, Cell 66:233-243, 1991; Zacharchuk, *J Immunol* 145:4037-4045, 1990; Zamai *et al.*, *Cytometry* 14:891-897, 1993; Gorczyca *et al.*, *Internat J Oncol* 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica *et al.*, *Blood* 84:111-117, 1994; Fine *et al.*, *Cell Immunol* 155: 111-122, 1994; Galy *et al.*, *Blood* 85:2770-2778, 1995; Toki *et al.*, *Proc Nat Acad Sci USA* 88:7548-7551, 1991.

Hematopoiesis Regulating Activity

An MBSPX protein of the present invention are useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, *e.g.* in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (*i.e.*, traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vivo or ex-vivo (*i.e.*, in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson *et al.* Cellular Biology 15:141-151, 1995; Keller *et al.*, Molecular and Cellular Biology 13:473-486, 1993; McClanahan *et al.*, Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M. G. In CULTURE OF HEMATOPOIETIC CELLS. Freshney, *et al.* eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, N.Y 1994; Hirayama *et al.*, *Proc Natl Acad Sci USA* 89:5907-5911, 1992; McNiece and Briddeli. In CULTURE OF HEMATOPOIETIC CELLS. Freshney, *et al.* eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, N.Y. 1994; Neben *et al.*, *Exp Hematol* 22:353-359, 1994; Ploemacher In CULTURE OF HEMATOPOIETIC CELLS. Freshney, *et al.* eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, N.Y. 1994; Spoonceret *al.*, In CULTURE OF HEMATOPOIETIC CELLS. Freshhey, *et al.* eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, N.Y. 1994; Sutherland, In CULTURE OF HEMATOPOIETIC CELLS. Freshney, *et al.* eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, N.Y. 1994.

Tissue Growth Activity

An MBSPX protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma

induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendonitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a career as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.* for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders,

which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International

Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, *Epidermal Wound Healing*, pp. 71-112 (Maibach, H I and Rovee, D T, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Menz, J. Invest. Dermatol 71:382-84 (1978).

Activin/Inhibin Activity

An MBSPX protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin a family, are useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin-b group, are useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, U.S. Pat. No. 4,798,885. A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale *et al.*, Endocrinology 91:562-572, 1972; Ling *et al.*, Nature 321:779-782, 1986; Vale *et al.*, Nature 321:776-779, 1986; Mason *et al.*, Nature 318:659-663, 1985; Forage *et al.*, *Proc Natl Acad Sci USA* 83:3091-3095, 1986.

Chemotactic/Chemokinetic Activity

A protein of the present invention may have chemotactic or chemokinetic activity (*e.g.*, act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured by following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: CURRENT PROTOCOLS IN IMMUNOLOGY, Coligan *et al.*, eds. (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28); Taub *et al.* J Clin Invest 95:1370-1376, 1995; Lind *et al.* APMIS 103:140-146, 1995; Muller *et al* Eur *J Immunol* 25: 1744-1748; Gruber*et al.* *J Immunol* 152:5860-5867, 1994; Johnston *et al.* *J Immunol* 153: 1762-1768, 1994.

Hemostatic and Thrombolytic Activity

A protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders

(including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (*e.g.*, stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet *et al.*, J. Clin. Pharmacol. 26:131-140, 1986; Burdick *et al.*, Thrombosis Res. 45:413-419, 1987; Humphrey *et al.*, Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

Receptor/Ligand Activity

A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell—cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in: Current Protocols in Immunology, Ed by J. *E. coli*gan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions

7.28.1-7.28.22), Takai *et al.*, *Proc Natl Acad Sci USA* 84:6864-6868, 1987; Bierer *et al.*, J. Exp. Med. 168:1145-1156, 1988; Rosenstein *et al.*, J. Exp. Med. 169:149-160 1989; Stoltenborg *et al.*, *J Immunol* Methods 175:59-68, 1994; Stitt *et al.*, Cell 80:661-670, 1995.

Anti-Inflammatory Activity

Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell—cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

Tumor Inhibition Activity

In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities. A protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth.

Other Activities

A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or circadian cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

EXAMPLES

Example 1. Molecular Cloning of 10354784.0.335.S3347a (MBSP2)

Oligonucleotide primers were designed to PCR amplify a DNA segment coding for a mature form of extracellular 10354784.0.335 from residues 26-653. The forward primer includes an in frame BglII restriction site and the reverse primer contains an in frame XhoI restriction site. The sequences of the primers are the following:

10354784 Mat F: AGA TCT GAG GCT GCC CGC ATC ATC TAC CCC CCA GAG (SEQ ID NO:23)

10354784 Rev: CTC GAG GCG AGC CAC CAT GGC CCC AGT GCC (SEQ ID NO:24).

PCR reactions were set up using 5 ng cDNA template derived from human kidney, pituitary and heart tissues, 1 microM of each of 10354784 Mat F and 10354784 Rev primers, 5 micromoles dNTP (Clontech Laboratories, Palo Alto CA) and 1 microliter of 50xAdvantage-HF 2 polymerase (Clontech Laboratories, Palo Alto CA) in 50 microliter volume. The following reaction conditions were used:

a) 96°C 3 minutes

b) 96°C 30 seconds denaturation

c) 70°C 30 seconds, primer annealing. This temperature was gradually decreased by 1°C/cycle

d) 72°C 3 minute extension.

Repeat steps b-d 10 times

e) 96°C 30 seconds denaturation

f) 60°C 30 seconds annealing

g) 72°C 3 minute extension

Repeat steps e-g 25 times

h) 72°C 5 minutes final extension

All three reactions resulted in the expected PCR product of about 1.8 kb detected by agarose gel electrophoresis. The kidney derived product was isolated and ligated into the pCR2.1 vector

(Invitrogen Corp, Carlsbad) and sequenced using vector specific primers and the following gene specific primers:

10354784 S1:	TCC GTG CTG TGG CTG AGG	(SEQ ID NO:25),
10354784 S2:	CCT CAG CCA CAG CAC GGA	(SEQ ID NO:26),
10354784 S3:	ACC TCC AAG ACA GAC TCA TAT	(SEQ ID NO:27),
10354784 S4:	ATA TGA GTC TGT CTT GGA GGT	(SEQ ID NO:28),
10354784 S5:	ACG GCC TCC GAG ACC TCA	(SEQ ID NO:29),
10354784 S6:	TGA GGT CTC GGA GGC CGT	(SEQ ID NO:30),
10354784 S7:	CC ACA GAC AGT GAC AAT G	(SEQ ID NO:31) and
10354784 S8:	CATT GTC ACT GTC TGT GG	(SEQ ID NO:32).

The construct is called pCR2.1-cg10354784.0.335-S334-7A. The cloned insert was determined as an ORF coding for a polypeptide having 628 amino acid residues, and is shown below in Table 3. There are 6 nucleotide changes compared to the sequence of clone 10354784.0.335 (SEQ ID NO:1) . The nucleotide changes result in three changes in the amino acid sequence (shown in Table 4) compared to the polypeptide encoded by clone 10354784.0.335 (SEQ ID NO:2). The other three nucleotide changes are silent mutations that do not change the amino acid sequence.

Table 3. DNA Sequence Analysis of 10354784.0.335.S334-7a

GAGGCTGCCCGCATCATCTACCCCCCAGAGGCCCAAAC
CATCATTGTCACCAAAGGCCAGAGTCTCATTCTGGAGTGTGTGGCCAGTGGAATCCCACCCCCACGGGTC
ACCTGGGCCAAGGATGGGTCCAGTGTCACCGGCTACAACAAGACGCGCTTCCTGCTGAGCAACCTCCTCA
TCGACACCACCAGCGAGGAGGACTCAGGCACCTCCCGGTGCATGCCCGACAATGGGGTTGGGCAGCCCGG
GGCAGCGGTCATCCTCTACAATGTCCAGGTGTTTGAACCCCCTGAGGTCACCATGGAGCTATCCCAGCTG
GTCATCCCCTGGGGCCAGAGTGCCAAGCTTACCTGTGAGGTGCGTGGGAACCCCCCGCCCTCCGTGCTGT
GGCTGAGGAATGCTGTGCCCCTCATCTCCAGCCAGCGCCTCCGGCTCTCCCGCAGGGCCCTGCGCGTGCT
CAGCATGGGGCCTGAGGACGAAGGCGTCTACCAGTGCATGGCCGAGAACGAGGTTGGGAGCGCCCATGCC
GTAGTCCAGCTGCGGACCTCCAGGCCAAGCATAACCCCAAGGCTATGGCAGGATGCTGAGCTGGCTACTG
GCACACCTCCTGTATCACCCTCCAAACTCGGCAACCCTGAGCAGATGCTGAGGGGGCAACCGGCGCTCCC
CAGACCCCCAACGTCAGTGGGGCCTGCTTCCCCGCAGTGTCCAGGAGAGAAGGGGCAGGGGGCTCCCGCC
GAGGCTCCCATCATCCTCAGCTCGCCCCGCACCTCCAAGACAGACTCATATGAACTGGTGTGGCGGCCTC
GGCATGAGGGCAGTGGCCGGGCGCCAATCCTCTACTATGTGGTGAAACACCGCAAGGTCACAAATTCCTC
TGACGATTGGACCATCTCTGGCATTCCAGCCAACCGGCACCGCCTGACCCTCACCAGACTTGACCCCGGG
AGCTTGTATGAAGTGGAGATGGCAGCTTACAACTGTGCGGGAGAGGGCCAGACAGCCATGGTCACCTTCC

GAACTGGACGGCGGCCCAAACCCGAGATCATGGCCAGCAAAGAGCAGCAGATCCAGAGAGACGACCCTGG
AGCCAGTCCCCAGAGCAGCAGTCCCCAGAGCAGCAGCCAGCCAGACCACGGCCGCCTCTCCCCCCCAGAA
GCTCCCGACAGGCCCACCATCTCCACGGCCTCCGAGACCTCAGTGTACGTGACCTGGATTCCCCGTGGGA
ATGGTGGGTTCCCAATCCAGTCCTTCCGTGTGGAGTACAAGAAGCTAAAGAAAGTGGGAGACTGGATTCT
GGCCACCAGCGCCATCCCCCCATCGCGGCTGTCCGTGGAGATCACGGGCCTAGAGAAAGGAGCCTCCTAC
AAGTTTCGAGTCCGGGCTCTGAACATGCTGGGGGAGAGCGAGCCCAGCGCCCCCTCTCGGCCCTACGTGG
TGTCGGGCTACAGCGGTCGCGTGTACGAGAGGCCCGTGGCAGGTCCTTATATCACCTTCACGGATGCGGT
CAATGAGACCACCATCATGCTCAAGTGGATGTACATCCCAGCAAGTAACAACAACACCCCAATCCATGGC
TTTTATATCTATTATCGACCCACAGACAGTGACAATGATAGTGACTACAAGAAGGATATGGTGGAAGGGG
ACAAGTACTGGCACTCCATCAGCCACCTGCAGCCAGAGACCTCCTACGACATTAAGATGCAGTGCTTCAA
TGAAGGAGGGGAGAGCGAGTTCAGCAACGTGATGATCTGTGAGACCAAAGCTCGGAAGTCTTCTGGCCAG
CCTGGTCGACTGCCACCCCCAACTCTGGCCCCACCACAGCCGCCCCTTCCTGAAACCATAGAGCGGCCGG
TGGGCACTGGGGCCATGGTGGCTCGC (SEQ ID NO:3)

TABLE 4. PREDICTED AMINO ACID SEQUENCE OF 10354784.0.335.S334-7A

EAARIIYPPEAQTIIVTKGQSLILECVASGIPPPRVTWAKDGSSVTGYNKTRFLLSNL
LIDTTSEEDSGTSRCMPDNGVGQPGAAVILYNVQVFEPPEVTMELSQLVIPWGQSAKLTCEVR
GNPPPSVLWLRNAVPLISSQRLRLSRRALRVLSMGPEDEGVYQCMAENEVGSAHAVVQLRTSR
PSITPRLWQDAELATGTPPVSPSKLGNPEQMLRGQPALPRPPTSVGPASPQCPGEKGQGAPAE
APIILSSPRTSKTDSYELVWRPRHEGSGRAPILYYVVKHRKVTNSSDDWTISGIPANRHRLTL
TRLDPGSLYEVEMAAYNCAGEGQTAMVTFRTGRRPKPEIMASKEQQIQRDDPGASPQSSSPQS
SSQPDHGRLSPPEAPDRPTISTASETSVYVTWIPRGNGGFPIQSFRVEYKKLKKVGDWILATS
AIPPSRLSVEITGLEKGASYKFRVRALNMLGESEPSAPSRPYVVSGYSGRVYERPVAGPYITF
TDAVNETTIMLKWMYIPASNNNTPIHGFYIYYRPTDSDNDSDYKKDMVEGDKYWHSISHLQPE
TSYDIKMQCFNEGGESEFSNVMICETKARKSSGQPGRLPPPTLAPPQPPLPETIERPVGTGAM
VAR (SEQ ID NO:4)

As noted in the Detailed Description of the Invention, the nucleotide sequence of clone 10354784.0.335 was searched against the GenBank databases using BLASTN search protocols. The BLASTN search showed that sequence 10354784.0.335 has 71% identity (450 of 632 nucleotides) to human CDO mRNA, 3986 bp (GenBank Accession No:AF004841).

Searches in publicly available GenBank database BLASTP showed that the protein encoded in related sequence 10354784.0.335 has 50% identity (265 of 525 residues) with ACC:O35158 rat CDO protein (1256 aa) and 50% identity (259 of 518 residues) with ACC:O14631 human CDO (1240 aa). CDO is an oncogene-, serum-, and anchorage-regulated member of the Ig/fibronectin type III repeat family (Kang et al., J. Cell Biol. 138 (1), 203-213 (1997)). Based upon homology, 10354784.0.335 and 10354784.0.335.S3347A proteins and each homologous protein or peptide may share at least some activity.

Example 2. Preparation of mammalian expression vector pCEP4/Sec

The oligonucleotide primers,

pSec-V5-His Forward: CTCGTCCTCGAGGGTAAGCCTATCCCTAAC (SEQ ID NO:33) and

pSec-V5-His Reverse: CTCGTCGGGCCCCTGATCAGCGGGTTTAAAC (SEQ ID NO:34)

were designed to amplify a fragment from the pcDNA3.1-V5His (Invitrogen, Carlsbad, CA) expression vector that includes V5 and His6. The PCR product was digested with XhoI and ApaI and ligated into the XhoI/ApaI digested pSecTag2 B vector harboring an Ig kappa leader sequence (Invitrogen, Carlsbad CA). The correct structure of the resulting vector, pSecV5His, including an in-frame Ig-kappa leader and V5-His6 was verified by DNA sequence analysis. The vector pSecV5His was digested with PmeI and NheI to provide a fragment retaining the above elements in the correct frame. The PmeI-NheI fragment was ligated into the BamHI/Klenow and NheI treated vector pCEP4 (Invitrogen, Carlsbad, CA). The resulting vector was named pCEP4/Sec and includes an in-frame Ig kappa leader, a site for insertion of a clone of interest, V5 and His6 under control of the PCMV and/or the PT7 promoter. pCEP4/Sec is an expression vector that allows heterologous protein expression and secretion by fusing any protein to the Ig Kappa chain signal peptide. Detection and purification of the expressed protein are aided by the presence of the V5 epitope tag and 6xHis tag at the C-terminus (Invitrogen, Carlsbad, CA).

Example 3. Expression of h10354784 in human embryonic kidney 293 cells.

The BglII-XhoI fragment containing the h10354784 sequence was isolated from pCR2.1-cg10354784-S334-7A and subcloned into the vector pCEP4/Sec (Example 2) to generate expression vector pCEP4/Sec-10354784. The pCEP4/Sec-10354784 vector was transfected into 293 cells using the LipofectaminePlus reagent following the manufacturer's

instructions (Gibco/BRL/Life Technologies, Rockville, MD). The cell pellet and supernatant were harvested 72 hours after transfection and examined for 10354784 expression by Western blotting under reducing conditions with an anti-V5 antibody. Fig. 1 shows that h10354784 is expressed as multiple sized polypeptides with a main product having an apparent molecular weight (Mr) of about 70 kDa secreted into the supernatant by 293 cells. This is close to the predicted value of 68794.1 Da.

Example 4. Expression of 20604798 in human embryonic kidney 293 cells.

The BglII-XhoI fragment containing the h20604798 sequence was isolated from pCR2.1-cg20604798-S319-2D and subcloned into the vector pCEP4/Sec (Example 2) to generate expression vector pCEP4/Sec-20604798. The pCEP4/Sec-20604798 vector was transfected into 293 cells using the LipofectaminePlus reagent following the manufacturer's instructions (Gibco/BRL/Life Technologies). The cell pellet and supernatant were harvested 72 hours after transfection and examined for 20604798 expression by Western blotting under reducing conditions with an anti-V5 antibody. Fig. 2 shows that 20604798 is expressed as two polypeptides secreted into the supernatant by the 293 cells, a less intense band whose Mr is about 62 kDa, and a main product having an Mr of about 53 kDa. The molecular weight standard used was SeeBlue Marker (Invitrogen, Carlsbad, CA).

Example 5. Expression of 3207791 in human embryonic kidney 293 cells.

The BamHI-XhoI fragment containing the 3207791 sequence was isolated from pCR2.1-cg3207791-S320-3E and subcloned into the vector pCEP4/Sec (Example 2) to generate expression vector pCEP4/Sec-3207791. The pCEP4/Sec-3207791 vector was transfected into 293 cells using the LipofectaminePlus reagent following the manufacturer's instructions (Gibco/BRL/Life Technologies). The cell pellet and supernatant were harvested 72 hours after transfection and examined for h3207791 expression by Western blotting under reducing conditions with an anti-V5 antibody. Fig. 3 shows that 3207791 is expressed as many polypeptides secreted into the supernatant by 293 cells, with a main product having an Mr of

apMBSPXimately 80 kDa. The molecular weight standard used was SeeBlue Marker (Invitrogen, Carlsbad, CA).

Example 6. Molecular Cloning of the Mature Form of Clone 20604798.0.1 (MBSP10)

The predicted open reading frame clone 20604798.0.1 codes for a 483 amino acid residue secreted protein. The cDNA coding for the mature form, predicted to contain residues 34-483, was targeted for cloning. Oligonucleotide primers were designed to PCR amplify a DNA segment coding for this polypeptide. The forward primer includes an in-frame BamHI restriction site and the reverse primer contains an in frame XhoI restriction site. The sequences of the primers are the following:

20604798 Forw: CTCGTCGGATCCCTTCCTCAACTCAGCGATGACATCC (SEQ ID NO:35) and

20604798 Rev: CTCGTCCTCGAGGTTGGGGAGAGAAAGAAGTCC (SEQ ID NO:36).

PCR reactions were set up using 5 ng cDNA template derived from human pituitary, 1 microM of each of 20604798 Forw and 20604798 Rev primers, 5 micromoles dNTP (Clontech Laboratories, Palo Alto CA) and 1 microliter of 50xAdvantage-HF 2 polymerase (Clontech Laboratories, Palo Alto CA) in 50 microliter volume. The reaction conditions used were the same as described in Example 1.

The major product band detected by agarose gel electrophoresis, having a size of apMBSPXimately 1350bp was isolated and ligated into the pCR2.1 vector (Invitrogen Corp, Carlsbad). The DNA was sequenced using vector derived primers and the following gene-specific primers:

20604798 S1: TACAGAATTGAGTCTTATTGG (SEQ ID NO:37),

20604798 S2: CCAATAAGACTCAATTCTGTA (SEQ ID NO:38),

20604798 S3: TCAGTAGCTGAAGTTACAACT (SEQ ID NO:39),

20604798 S4: AGTTGTAACTTCAGCTACTGA (SEQ ID NO:40),

20604798 S5: GGTTCTTACTGCTTTCGTGGG (SEQ ID NO:41),

20604798 S6: CCCACGAAAGCAGTAAGAACC (SEQ ID NO:42),

The cloned construct is called pCR2.1-20604798-S319-2d. This insert was determined to be an ORF that matches the sequence of clone 20604798.0.1 exactly (SEQ ID NO:19), and codes for the expected 450 residue polypeptide (SEQ ID NO:20).

Example 7. Molecular Cloning of the Mature Form of Clone 3207791.0.128 (MBSP6)

The predicted open reading frame for clone 3207791.0.128 codes for a 535 amino acid secreted protein. The cDNA coding for the predicted mature form spanning residues 23-535 was targeted for cloning. Oligonucleotide primers were designed to PCR amplify this DNA sequence. The forward primer includes an in frame BamHI restriction site and the reverse primer contains an in-frame XhoI restriction site. The sequences of the primers are the following:

3207791 Forward: CTCGTCGGATCCGAGTCTGAGACTGGGCCCATGGAGG (SEQ ID NO:43), and

3207791 Reverse: CTCGAGGCCCCGGTGGTGGTGGTGGTGGCTATGGCTG (SEQ ID NO:44)

PCR reactions were set up using 5 ng cDNA template derived from human pituitary, 1 microM of each of 20604798 Forw and 20604798 Rev primers, 5 micromoles dNTP (Clontech Laboratories, Palo Alto CA) and 1 microliter of 50xAdvantage-HF 2 polymerase (Clontech Laboratories, Palo Alto CA) in 50 microliter volume. The reaction conditions used were the same as described in Example 1.

One major, 1500bp large, amplified product was detected by agarose gel electrophoresis. The product was isolated and ligated into the pCR2.1 vector (Invitrogen Corp, Carlsbad). The DNA sequenced using vector derived and the following genespecific primers:

3207791 S1:	GAAGCCTTCCAGCGGGCTCTG	(SEQ ID NO:45),
3207791 S2:	CAGAGCCCGCTGGAAGGCTTC	(SEQ ID NO:46),
3207791 S3:	GGCTTGGCTGAGCTGGGCCAC	(SEQ ID NO:47),
3207791 S4:	GTGGCCCAGCTCAGCCAAGCC	(SEQ ID NO:48),
3207791 S5:	CGACAGGGCCCGAAGGAACC	(SEQ ID NO:49),
3207791 S6:	GGTTCCTTCGGGCCCTGTCG	(SEQ ID NO:50),
3207791 S7:	GCAGCTGACCAAGGAGCTAC	(SEQ ID NO:51), and
3207791 S8:	GTAGCTCCTTGGTCAGCTGC	(SEQ ID NO:52).

The cloned construct is called pCR2.1-cg3207791.0.128-pCR2.1-S320-3E. The insert was determined as an ORF coding for a polypeptide of 513 amino acids.

The nucleotide sequence of the insert in pCR2.1-cg3207791.0.128-pCR2.1-S320-3E is shown in Table 5. It differs at two positions, shown in bold font, from the corresponding sequence of clone 3207791.0.128:

Table 5

GAGTCTGAGACTGGGCCCATGGAGGAAGTGGAGCGGCAGGTCCTCCCAGACCCCGAGGTGCTGGAAGCT
GTGGGGGACAGGCAGGATGGGCTAAGGGAACAGCTGCAGGCCCCAGTGCCTCCTGACAGTGTCCCCAGC
CTGCAAAACATGGGTCTTCTGCTGGACAAGCTGGCCAAGGAGAACCAGGACATCCGGCTGCTGCAGGCC
CAGCTGCAGGCCCAAAAGGAAGAGCTTCAGAGCCTGATGCACCAGCCCAAAGGGCTAGAGGAGGAGAAT
GCCCAGCTCCGGGGGGCTCTGCAGCAGGGCGAAGCCTTCCAGCGGGCTCTGGAGTCAGAGCTGCAGCAG
CTGCGGGCCCGGCTCCAGGGGCTGGAGGCCGACTGTGTCCGGGGCCCAGATGGGGTGTGCCTCAGTGGG
GATAGAGGCCCACAGGGTGACAAGGCCATCAGGGAGCAAGGCCCCAGGGAGCAGGAGCCAGAACTCAGC
TTCCTGAAGCAGAAGGAACAGCTGGAGGCTGAGGCACAGGCATTAAGGCAAGAGTTAGAGAGGCAGCGA
CGGCTGCTGGGGTCTGTACAGCAGGATCTGGAGAGGAGCTTGCAGGATGCCAGCCGCGGGGACCCAGCT
CATGCTGGCTTGGCTGAGCTGGGCCACAGATTGGCCCAGAAACTGCAGGGCCTGGAGAACTGGGGCCAG
GACCCTGGGGTCTCTGCCAATGCCTCAAAGGCCTGGCACCAGAAGTCCCACTTCCAGAATTCTAGGGAG
TGGAGTGGAAAGGAAAAGTGGTGGGATGGGCAGAGAGACCGGAAGGCTGAGCACTGGAAACATAAGAAG
GAAGAATCTGGCCGGGAAAGGAAGAAGAACTGGGGAGGTCAGGAGGACAGGGAGCCAGCAGGAAGGTGG
AAGGAGGGCAGGCCAAGGGTGGAGGAGTCGGGGAGCAAGAAGGAGGGCAAGCGACAGGGCCCGAAGGAA
CCCCCAAGGAAAAGTGGTAGCTTCCACTCCTCTGGAGAAAAGCAGAAGCAACCTCGGTGGAGGGAAGGG
ACTAAGGACAGCCATGACCCCCTGCCATCCTGGGCAGAGCTGTTGAGGCCCAAGTACCGGGCACCCCAG
GGCTGCTCAGGTGTGGACGAGTGTGCCCGGCAGGAGGGCCTGACTTTCTTTGGCACAGAGCTAGCCCCA
GTGCGGCAACAGGAGCTGGCCTCTCTGCTAAGAACATACTTGGCACGGCTGCCCTGGGCTGGGCAGCTG

ACCAAGGAGCTACCCCTCTCACCTGCTTTCTTTGGTGAGGATGGCATCTTCCGTCATGACCGCCTCCGC
TTCCGGGATTTTGTGGATGCCCTGGAGGACAGCTTGGAGGAGGTGGCTGTGCAACAGACAGGTGATGAT
GATGAAGTAGATGACTTTGAGGACTTCATCTTCAGCCACTTCTTTGGAGACAAAGCACTGAAGAAGAGG
TCAGGGAAGAAGGACAAGCACTCACAGAGCCCAAGAGCTGCGGGGCCCAGGGAGGGGCACAGCCATAGC
CACCACCACCACCACCGGGGC
(SEQ ID NO:11).

The amino acid sequence of the polypeptide encoded by the insert in pCR2.1-cg3207791.0.128-pCR2.1-S320-3E is shown in Table 6. The two nucleotide differences shown in Table 5 translate to two amino acid differences in the polypeptide.

Table 6

ESETGPMEEVERQVLPDPEVLEAVGDRQDGLREQLQAPVPPDSVPSLQNMGLLLDKL
AKENQDIRLLQAQLQAQKEELQSLMHQPKGLEEENAQLRGALQQGEAFQRALESELQQLRAR
LQGLEADCVRGPDGVCLSGDRGPQGDKAIREQGPREQEPELSFLKQKEQLEAEAQALRQELER
QRRLLGSVQQDLERSLQDASRGDPAHAGLAELGHRLAQKLQGLENWGQDPGVSANASKAW
HQKSHFQNSREWSGKEKWWDGQRDRKAEHWKHKKEESGRERKKNWGGQEDREPAGRWKE
GRPRVEESGSKKEGKRQGPKEPPRKSGSFHSSGEKQKQPRWREGTKDSHDPLPSWAELLRPKY
RAPQGCSGVDECARQEGLTFFGTELAPVRQQELASLLRTYLARLPWAGQLTKELPLSPAFFGE
DGIFRHDRLRFRDFVDALEDSLEEVAVQQTGDDDEVDDFEDFIFSHFFGDKALKKRSGKKDKH
SQSPRAAGPREGHSHSHHHHHRG (SEQ ID NO:12).

Example 8. Real Time Expression Analysis

The quantitative expression of various clones was assessed in about 41 normal and about 55 tumor samples by real time quantitative PCR (TAQMAN®) performed on a Perkin-Elmer Biosystems ABI PRISM® 7700 Sequence Detection System.

First, 96 RNA samples were normalized to β-actin and GAPDH. RNA (~50 ng total or ~1 ng polyA+) was converted to cDNA using the TAQMAN® Reverse Transcription Reagents Kit (PE Biosystems, Foster City, CA; cat # N808-0234) and random hexamers according to the manufacturer's protocol. Reactions were performed in 20 ul and incubated for 30 min. at 48°C. cDNA (5 ul) was then transferred to a separate plate for the TAQMAN® reaction using β-actin and GAPDH TAQMAN® Assay Reagents (PE Biosystems; cat. #'s 4310881E and 4310884E, respectively) and TAQMAN® universal PCR Master Mix (PE Biosystems; cat #

4304447) according to the manufacturer's protocol. Reactions were performed in 25 ul using the following parameters: 2 min. at 50°C; 10 min. at 95°C; 15 sec. at 95°C/1 min. at 60°C (40 cycles). Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100. The average CT values obtained for ß-actin and GAPDH were used to normalize RNA samples. The RNA sample generating the highest CT value required no further diluting, while all other samples were diluted relative to this sample according to their β-actin /GAPDH average CT values.

Normalized RNA (5 ul) was converted to cDNA and analyzed via TAQMAN® using One Step RT-PCR Master Mix Reagents (PE Biosystems; cat. # 4309169) and gene-specific primers according to the manufacturer's instructions. Probes and primers were designed for each assay according to Perkin Elmer Biosystem's *Primer Express* Software package (version I for Apple Computer's Macintosh Power PC) using the sequence of the target clone as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration = 250 nM, primer melting temperature (T_m) range = 58°-60° C, primer optimal Tm = 59° C, maximum primer difference = 2° C, probe does not have 5' G, probe T_m must be 10° C greater than primer T_m, amplicon size 75 bp to 100 bp. The probes and primers selected (see below) were synthesized by Synthegen (Houston, TX, USA). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900 nM each, and probe, 200nM.

PCR conditions: normalized RNA from each tissue and each cell line was spotted in each well of a 96 well PCR plate (Perkin Elmer Biosystems). PCR cocktails including two probes (SEQX-specific and another gene-specific probe multiplexed with the SEQX probe) were set up using 1X TaqMan™ PCR Master Mix for the PE Biosystems 7700, with 5 mM MgCl2, dNTPs (dA, G, C, U at 1:1:1:2 ratios), 0.25 U/ml AmpliTaq Gold™ (PE Biosystems), and 0.4 U/µl RNase inhibitor, and 0.25 U/µl reverse transcriptase. Reverse transcription was

performed at 48° C for 30 minutes followed by amplification/PCR cycles as follows: 95° C 10 min, then 40 cycles of 95° C for 15 seconds, 60° C for 1 minute.

The primer probe set Ag 70 was used to detect the expression of clone 17939072.0.47 (MBSP3) in the various tissues.

Forward:	ACCGTGACAGCGACCATTC	(SEQ ID NO:53)
Reverse:	GTGTGGCAGTTGCGGTACC	(SEQ ID NO:54)
Probe:	Fam-AACTGTGCCGCCTTCTACCGCG-Tamra	(SEQ ID NO:55)

The results are presented in Table 7. It is seen that clone 17939072.0.47 (MBSP3) is expressed in many cancer cell lines but not in the cognate normal cell lines.

Table 7

Normal & Tumor Tissues	Relative Expression (%)	Normal & Tumor Tissues	Relative Expression (%)
Endothelial cells	0.00	Renal ca. 786-0	5.33
Endothelial cells (treated)	0.00	Renal ca. A498	63.29
Pancreas	0.00	Renal ca. RXF 393	28.13
Pancreatic ca. CAPAN 2	3.47	Renal ca. ACHN	2.12
Adipose	1.53	Renal ca. UO-31	0.48
Adrenal gland	24.66	Renal ca. TK-10	0.48
Thyroid	0.65	Liver	0.00
Salivary gland	0.13	Liver (fetal)	0.00
Pituitary gland	0.06	Liver ca. (hepatoblast) HepG2	3.00
Brain (fetal)	3.93	Lung	0.28
Brain (whole)	3.30	Lung (fetal)	1.88
Brain (amygdala)	23.98	Lung ca. (small cell) LX-1	87.06
Brain (cerebellum)	0.38	Lung ca. (small cell) NCI-H69	14.97

Brain (hippocampus)	100.00	Lung ca. (s.cell var.) SHP-77	17.08
Brain (hypothalamus)	0.24	Lung ca. (large cell)NCI-H460	60.71
Brain (substantia nigra)	0.98	Lung ca. (non-sm. cell) A549	12.50
Brain (thalamus)	30.57	Lung ca. (non-s.cell) NCI-H23	0.90
Spinal cord	0.43	Lung ca (non-s.cell) HOP-62	0.82
CNS ca. (glio/astro) U87-MG	0.78	Lung ca. (non-s.cl) NCI-H522	12.07
CNS ca. (glio/astro) U-118-MG	0.17	Lung ca. (squam.) SW 900	12.24
CNS ca. (astro) SW1783	11.50	Lung ca. (squam.) NCI-H596	2.11
CNS ca.* (neuro; met) SK-N-AS	0.06	Mammary gland	1.81
CNS ca. (astro) SF-539	37.89	Breast ca.* (pl. effusion) MCF-7	38.96
CNS ca. (astro) SNB-75	4.07	Breast ca.* (pl.ef) MDA-MB-231	1.18
CNS ca. (glio) SNB-19	19.89	Breast ca.* (pl. effusion) T47D	12.33
CNS ca. (glio) U251	61.99	Breast ca. BT-549	48.63
CNS ca. (glio) SF-295	2.02	Breast ca. MDA-N	0.03
Heart	0.47	Ovary	0.10
Skeletal muscle	0.00	Ovarian ca. OVCAR-3	0.21
Bone marrow	0.00	Ovarian ca. OVCAR-4	0.03
Thymus	0.00	Ovarian ca. OVCAR-5	30.15
Spleen	0.15	Ovarian ca. OVCAR-8	26.06
Lymph node	0.03	Ovarian ca. IGROV-1	0.07
Colon (ascending)	0.96	Ovarian ca.* (ascites) SK-OV-3	1.73
Stomach	0.08	Myometrium	0.75
Small intestine	1.09	Uterus	0.17
Colon ca. SW480	2.61	Placenta	1.50
Colon ca.* (SW480 met)SW620	30.99	Prostate	0.35
Colon ca. HT29	0.23	Prostate ca.* (bone met)PC-3	15.60
Colon ca. HCT-116	36.86	Testis	57.04

Colon ca. CaCo-2	0.49	Melanoma Hs688(A).T	0.26
Colon ca. HCT-15	7.48	Melanoma* (met) Hs688(B).T	1.07
Colon ca. HCC-2998	1.28	Melanoma UACC-62	0.14
Gastric ca.* (liver met) NCI-N87	35.11	Melanoma M14	0.20
Bladder	0.11	Melanoma LOX IMVI	1.10
Trachea	0.30	Melanoma* (met) SK-MEL-5	0.51
Kidney	0.06	Melanoma SK-MEL-28	0.11
Kidney (fetal)	1.05	Melanoma UACC-257	0.59

ca. = carcinoma

* = established from metastasis

met = metastasis

s cell var= small cell variant

non-s = non-sm =non-small

squam = squamous

pl. eff = pl effusion = pleural effusion

glio = glioma

astro = astrocytoma

neuro = neuroblastoma

Example 9. Radiation Hybrid Mapping Identifies the Chromosomal Location of Clones of the Invention.

Radiation hybrid mapping using human chromosome markers was carried out for many of the clones described in the present invention. The procedure used to obtain these results is analogous to that described in Steen, RG et al. (A High-Density Integrated Genetic Linkage and Radiation Hybrid Map of the Laboratory Rat, Genome Research 1999 (Published Online on May 21, 1999) Vol. 9, AP1-AP8, 1999). Detailed description of RH mapping may be found in, *e.g.*, Cox *et al.* (1990) *Science* 250: 245-250; Boehnke *et al.* (1991) *Am J Hum Genet* 49: 1174-1188; and Walter *et al.* (1994) *Nat Genet* 7: 22-28.

A panel of 93 cell clones containing randomized radiation-induced human

chromosomal fragments was screened in 96 well plates using PCR primers designed to identify the sought clones in a unique fashion. Table 8 provides the results obtained for three clones of the present invention, showing the markers straddling the gene of the invention, and the distance in cR separating them.

Table 8

MBSPX Number	Clone	Chromosome	Distance from Marker, cR	Distance from Marker, cR
1	10354784.0.335	3	WI-1780, 0.0	--
9	16401346.0.337	1	WI-611, 4.1	D1S195, 6.7
10	20604798.0.1	2	WI-4077, 0.0	--
7	3499605.0.64	1	AFM311ZG1, 11.6	CHLC.GATA31D10, 5.1

OTHER EMBODIMENTS

Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims. In particular, it is contemplated by the inventor that various substitutions, alterations, and modifications may be made to the invention without departing from the spirit and scope of the invention as defined by the claims. Therefore, other aspects, advantages, and modifications are within the scope of the following claims.

What is claimed is:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

a) a mature form of the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22;

b) a variant of a mature form of the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, wherein any amino acid in the mature form is changed to a different amino acid, provided that no more than 15% of the amino acid residues in the sequence of the mature form are so changed;

c) the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22;

d) a variant of the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15% of the amino acid residues in the sequence are so changed; and

e) a fragment of any of a) through d).

2. The polypeptide of claim 1 that is a naturally occurring allelic variant of the sequence given by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22.

3. The polypeptide of claim 2, wherein the variant is the translation of a single nucleotide polymorphism.

4. The polypeptide of claim 1 that is a variant polypeptide described therein, wherein any amino acid specified in the chosen sequence is changed to provide a conservative substitution.

5. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of:

a) a mature form of the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22;

b) a variant of a mature form of the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 wherein any amino acid in the mature form of the chosen sequence is changed to a different amino acid, provided that no more than 15% of the amino acid residues in the sequence of the mature form are so changed;

c) the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22;

d) a variant of the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, in which any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15% of the amino acid residues in the sequence are so changed;

e) a nucleic acid fragment encoding at least a portion of a polypeptide comprising the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 or any variant of said polypeptide wherein any amino acid of the chosen sequence is changed to a different amino acid, provided that no more than 10% of the amino acid residues in the sequence are so changed; and

f) the complement of any of said nucleic acid molecules.

6. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises the nucleotide sequence of a naturally occurring allelic nucleic acid variant.

7. The nucleic acid molecule of claim 5 that encodes a variant polypeptide, wherein the variant polypeptide has the polypeptide sequence of a naturally occurring polypeptide variant.

8. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises a single nucleotide polymorphism encoding said variant polypeptide.

9. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of

a) the nucleotide sequence given by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21;

b) a nucleotide sequence wherein one or more nucleotides in the nucleotide sequence given by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21 is changed from that given by the chosen sequence to a different nucleotide provided that no more than 15% of the nucleotides are so changed;

c) a nucleic acid fragment of the sequence given by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21; and

d) a nucleic acid fragment wherein one or more nucleotides in the nucleotide sequence given by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21 is changed from that given by the chosen sequence to a different nucleotide provided that no more than 15% of the nucleotides are so changed.

10. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule hybridizes under stringent conditions to the nucleotide sequence given by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, or a complement of said nucleotide sequence.

11. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises a nucleotide sequence in which any nucleotide specified in the coding sequence of the chosen nucleotide sequence is changed from that given by the chosen sequence to a different nucleotide provided that no more than 15% of the nucleotides in the chosen coding sequence are so changed, an isolated second polynucleotide that is a complement of the first polynucleotide, or a fragment of any of them.

12. A vector comprising the nucleic acid molecule of claim 11.

13. The vector of claim 12, further comprising a promoter operably linked to said nucleic acid molecule.

14. A cell comprising the vector of claim 12.

15. An antibody that binds immunospecifically to the polypeptide of claim 1.

16. The antibody of claim 15, wherein said antibody is a monoclonal antibody.

17. The antibody of claim 15, wherein said antibody is a humanized antibody.

18. A method for determining the presence or amount of the polypeptide of claim 1 in a sample, the method comprising:

(a) providing said sample;

(b) introducing said sample to an antibody that binds immunospecifically to the polypeptide; and

(c) determining the presence or amount of antibody bound to said polypeptide,

thereby determining the presence or amount of polypeptide in said sample.

19. A method for determining the presence or amount of the nucleic acid molecule of claim 5 in a sample, the method comprising:

(a) providing said sample;

(b) introducing said sample to a probe that binds to said nucleic acid molecule; and

(c) determining the presence or amount of said probe bound to said nucleic acid molecule,

thereby determining the presence or amount of the nucleic acid molecule in said sample.

20. A method of identifying an agent that binds to the polypeptide of claim 1, the method comprising:

(a) introducing said polypeptide to said agent; and

(b) determining whether said agent binds to said polypeptide.

21. A method for identifying a potential therapeutic agent for use in treatment of a pathology, wherein the pathology is related to aberrant expression or aberrant physiological interactions of the polypeptide of claim 1, the method comprising:

(a) providing a cell expressing the polypeptide of claim 1 and having a property or function ascribable to the polypeptide;

(b) contacting the cell with a composition comprising a candidate substance; and

(c) determining whether the substance alters the property or function ascribable to the polypeptide;

whereby, if an alteration observed in the presence of the substance is not observed when the cell is contacted with a composition devoid of the substance, the substance is identified as a potential therapeutic agent.

22. A method for modulating the activity of the polypeptide of claim 1, the method comprising introducing a cell sample expressing the polypeptide of said claim with a compound that binds to said polypeptide in an amount sufficient to modulate the activity of the polypeptide.

23. A method of treating or preventing a pathology associated with the polypeptide of claim 1, said method comprising administering the polypeptide of claim 1 to a subject in which such treatment or prevention is desired in an amount sufficient to treat or prevent said pathology in said subject.

24. The method of claim 23, wherein said subject is a human.

25. A method of treating or preventing a pathology associated with the polypeptide of claim 1, said method comprising administering to a subject in which such treatment or prevention is desired an MBSPX nucleic acid in an amount sufficient to treat or prevent said pathology in said subject.

26. The method of claim 25, wherein said subject is a human.

27. A method of treating or preventing a pathology associated with the polypeptide of claim 1, said method comprising administering to a subject in which such treatment or prevention is desired an MBSPX antibody in an amount sufficient to treat or prevent said pathology in said subject.

28. The method of claim 27, wherein the subject is a human.

29. A pharmaceutical composition comprising the polypeptide of claim 1 and a pharmaceutically acceptable carrier.

30. A pharmaceutical composition comprising the nucleic acid molecule of claim 5 and a pharmaceutically acceptable carrier.

31. A pharmaceutical composition comprising the antibody of claim 15 and a pharmaceutically acceptable carrier.

32. A kit comprising in one or more containers, the pharmaceutical composition of claim 29.

33. A kit comprising in one or more containers, the pharmaceutical composition of claim 30.

34. A kit comprising in one or more containers, the pharmaceutical composition of claim 31.

35. A method for screening for a modulator of activity or of latency or predisposition to a pathology associated with the polypeptide of claim 1, said method comprising:

a) administering a test compound to a test animal at increased risk for a pathology associated with the polypeptide of claim 1, wherein said test animal recombinantly expresses the polypeptide of claim 1;

b) measuring the activity of said polypeptide in said test animal after administering the compound of step (a); and

c) comparing the activity of said protein in said test animal with the activity of said polypeptide in a control animal not administered said polypeptide, wherein a change in the activity of said polypeptide in said test animal relative to said control animal indicates the test compound is a modulator of latency of, or predisposition to, a pathology associated with the polypeptide of claim 1.

36. The method of claim 35, wherein said test animal is a recombinant test animal that expresses a test protein transgene or expresses said transgene under the control of a promoter at an increased level relative to a wild-type test animal, and wherein said promoter is not the native gene promoter of said transgene.

37. A method for determining the presence of or predisposition to a disease associated with altered levels of the polypeptide of claim 1 in a first mammalian subject, the method comprising:

a) measuring the level of expression of the polypeptide in a sample from the first mammalian subject; and

b) comparing the amount of said polypeptide in the sample of step (a) to the amount of the polypeptide present in a control sample from a second mammalian subject known not to have, or not to be predisposed to, said disease,

wherein an alteration in the expression level of the polypeptide in the first subject as compared to the control sample indicates the presence of or predisposition to said disease.

38. A method for determining the presence of or predisposition to a disease associated with altered levels of the nucleic acid molecule of claim 5 in a first mammalian subject, the method comprising:

a) measuring the amount of the nucleic acid in a sample from the first mammalian subject; and

b) comparing the amount of said nucleic acid in the sample of step (a) to the amount of the nucleic acid present in a control sample from a second mammalian subject known not to have or not be predisposed to, the disease;

wherein an alteration in the level of the nucleic acid in the first subject as compared to the control sample indicates the presence of or predisposition to the disease.

39. A method of treating a pathological state in a mammal, the method comprising administering to the mammal a polypeptide in an amount that is sufficient to alleviate the pathological state, wherein the polypeptide is a polypeptide having an amino acid sequence at least 95% identical to a polypeptide comprising the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 or a biologically active fragment thereof.

40. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the antibody of claim 15 in an amount sufficient to alleviate the pathological state.

Figure 1.

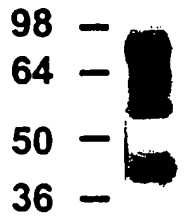

Figure 2.

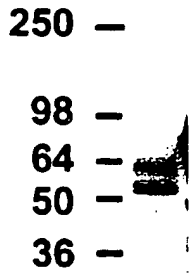

Figure 3.